THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 112 MAY 1, 1935 No. 1

PITCH AND INTENSITY DISCRIMINATION BY CATS

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Received for publication January 23, 1935

While much is known concerning the acoustic analyser of the dog (Pavlov, 1927), observations upon the cat have been few. Zeliony (1909) reported in one cat ability to distinguish between two notes of an organ pipe, half a tone apart. Shepherd (1914) states that cats can discriminate between notes one and two octaves apart.

In the present investigation an attempt was made to measure separately the sensitivity of the "acoustic analyser" of the cat to differences of intensity and of pitch of pure musical notes. A motor-alimentary training method was used throughout—see Dworkin (1934). During the experiments the animals were enclosed in a silence room entirely isolated from the observer. The pure musical notes were generated by means of a variable-frequency audio oscillator, and delivered by a large magnetic loud speaker placed about 1 foot from the animals. To control the loudness of the sounds, an attenuation meter calibrated in steps of 2 decibels was placed between the oscillator and loud-speaker.[1] By lighting the filament of an intermediate amplifier the sound stimuli could be turned on without accompanying clicks.

By a minimum of 200 tests the animals had all been previously trained to respond positively to a wide range of musical sounds.

The procedure used in establishing discriminations was that of "differentiating inhibition" (Pavlov). When a "positive" tone is sounded, the animal receives food, but not when a "negative" tone is sounded. Ultimately, owing to non-reinforcement, the response to the negative stimulus is abolished. Usually one, sometimes two, negative stimuli

[1] For each frequency, the output of the oscillator was set so that attenuation of 60 decibels reduced the loudness to the threshold of hearing in man. Hence a loudness of 60 decibels was produced by zero attenuation of the meter, a loudness of 10 decibels by 50 decibels' attenuation, etc.

were used per day, along with six or seven positive stimuli. The time interval between any two stimuli varied from two to five minutes.

I. *The intensity factor in pitch discrimination.* The first experiments brought out the importance of loudness in work on tone discrimination. In one cat perfect differentiation was obtained between two notes which differed not only in pitch but also in loudness. The intensity of the louder tone was then diminished, and the differentiation disappeared, although the frequency remained unaltered. On further study, it was found that whenever pitch discrimination was developed in the presence of a notable intensity difference, the significance of the notes could be reversed by reversing the intensities. For example, one cat was trained to respond positively to a frequency of 2600 cycles per second with a loudness of 60 decibels, and negatively to a frequency of 4000 cycles with a loudness of 30 decibels. Thereupon the loudness of the positive tone was reduced to about 30 decibels, and the cat received food several times while this quieter positive tone was presented. The animal remained positive. Later, when the previously negative tone, likewise of 30 decibels, was sounded, the response was now strongly positive.

Another cat was trained to respond positively to a note of 2600 cycles with a loudness of 60 decibels, and negatively to a note of 3500 cycles with a loudness of 30 decibels. Then the loudness of the 2600 cycle note was reduced to 30 decibels, whereupon the animal failed to respond. When the loudness was increased to 60 decibels, a strong positive response was again elicited.

II. *The limits of intensity discrimination by cats.* An attempt was next made to work out the limits of intensity discrimination in two cats. These animals were trained first to respond to a note of fixed frequency, and then to distinguish in that note differences of intensity.

In one cat the frequency chosen was 2600 cycles per second. For the first differentiations a loudness of 56 decibels was selected as the positive stimulus, that of 30 decibels as the negative stimulus. The distinction was made after 5 trials of the negative stimulus. At the 12th trial 48 decibels was differentiated from 30 decibels. At the 20th trial 54 decibels was distinguished from 38 decibels, and at the 23rd trial the differentiation between 54 decibels and 42 decibels was strong. From this point on the problem for the cat became more difficult. Only at the 56th trial was the animal able to distinguish 54 decibels from 48 decibels, and this distinction was at times uncertain and often absent. After 71 trials this cat was able to discriminate, at times, between 54 and 50 decibels, but the distinction was unstable. Closer differentiation, even after many further trials, was quite inconstant, so it seems reasonable to conclude that the limit of loudness discrimination in this cat was about 4 decibels.

In another cat a similar experiment was carried out, the tone-frequency

being this time 1500 cycles per second. Here the loudness of the positive signal was varied, while that of the negative was kept constant. In this cat also the limit of discrimination proved to be 4 decibels.

In both animals it was observed that, when the difference in loudness was 8 decibels or more, the differentiation was fixed or "absolute" (Pavlov), i.e., there never was any confusion on the part of the animal even when the louder of the two sounds was purposely preceded by a sound 8 decibels louder still. When the difference in loudness was less than 8 decibels, the cats occasionally mistook the negative for the positive stimulus, the confusion becoming frequent and very definite as the 4-decibel limit was exceeded. An animal that has been trained to respond positively to 54 decibels and negatively to 50 decibels, will respond negatively to 54 decibels if this signal is preceded by one of 58 or 60 decibels.

III. *Pitch discrimination in the cat.* For the work on pitch discrimination, three well trained, active cats were chosen.

A typical protocol of one of these experiments is as follows: To begin with, a note of 2000 cycles per second was chosen as the positive, a note of 3400 cycles as the negative stimulus. Complete differentiation was established at the 7th trial. The negative note was then stepped down to 3000 c.p.s. and by the 11th test this too was differentiated from 2000. Then the negative stimulus was brought down to 2900; this took 16 to 17 further trials, so that in a total of 27 to 28 negative tests differentiation between 2000 and 2900 c.p.s. was well established.

The negative note was then moved down to 2600; differentiation was complete (zero response to 2 successive negative stimuli). Now 2500 c.p.s. was made the negative stimulus. This differentiation was complete at the 50th negative test. At the 56th trial, 2360 c.p.s. was differentiated from 2000 c.p.s. Next the positive stimulus was moved down to 2150 c.p.s., so that the cat now had to distinguish 2360 c.p.s. from 2150 c.p.s. This differentiation was very difficult but was attained once at the 76th trial, was weakly evident at trial no. 77, and was really established only after the 84th test. Attempts to effect a closer differentiation were only occasionally successful.

A second cat was trained to discriminate between notes of 2500 and 2160 cycles per second in 65 tests, while a third cat was, in 77 tests, trained to distinguish a note of 2600 from 2900 cycles per second. In neither animal was it possible, even after 30 more trials, to form closer differentiations.

SUMMARY

By means of an alimentary-motor conditioning method, cats were trained to discriminate between musical notes differing in loudness and in pitch. The extreme limit of loudness differentiation was about 4 decibels, that of pitch discrimination about one tone. Whenever pitch discrimina-

tion was developed in the presence of a considerable difference in loudness, it was possible to reverse the significance of the notes by changing the intensity. Hence it is necessary when dealing only with pitch discrimination to use notes of equal loudness.

For financial assistance in this work, the author wishes to acknowledge his indebtedness to the Research Bureau of the American Otological Society.

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HEXOSEMONOPHOSPHATE CHANGES IN MUSCLE IN RELATION TO RATE OF STIMULATION AND WORK PERFORMED

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Received for publication January 28, 1935

An increase in the hexosemonophosphate content of muscle after various forms of stimulation has been consistently observed in this laboratory (1), but what factors might play a rôle in the accumulation of hexosephosphate have not been investigated in detail. It was noted that when a muscle was stimulated tetanically there seemed to be a greater increase in hexosephosphate than when the stimulation was effected by single shocks, and this occurred in spite of the fact that in some cases more lactic acid accumulated during single shock than during tetanic stimulation. Since these muscles were stimulated *in situ* no measurement of work was made. However, such findings suggested that the rate of stimulation might influence the accumulation of hexosephosphate and accordingly an investigation was carried out to test this possibility.

There exist a few reports in the literature on the effect of rate of stimulation on lactic acid formation in isolated frog muscle. Lundsgaard (2) and Meyerhof and Schulz (3) found no change in the $\frac{\text{lactic acid}}{\text{work}}$ ratio with single shocks applied at different rates, while Lenhartz (4) obtained slightly more lactic acid per unit of work with slow as compared to rapid rates of stimulation. Visscher and Smith (5) reported that lactic acid produced per unit of work varied inversely with the rate of stimulation, but the element of fatigue was not ruled out in their experiments.

EXPERIMENTAL. Gastrocnemii from large grass frogs of the same shipment were used throughout each series of experiments. After spinal transsection, the muscles were removed along with the femoral attachment and part of the sciatic nerve, placed in individual tubes containing 10 cc. of oxygenated Ringer's solution maintained at pH 7.2 with CO_2—$NaHCO_3$ buffer, and allowed to recover after dissection for two to three hours at 20°C. One muscle was then removed from the bath, suspended in a moist chamber containing N_2 at room temperature (20–25°C.), and affixed to the tension lever and electrodes, after which a tension of 25 to 30 grams was

applied, the muscle length taken, and the chamber closed. After stimulation the muscle was fixed in $HCl-HgCl_2$ solution contained in tared and stoppered tubes, as described in a previous paper (6). The mate to the muscle mentioned above was taken in some instances for the determination of resting levels and in others for stimulation at a different rate.

Analyses. Hexosemonophosphate hexose and phosphorus were determined as described elsewhere (7) and lactic acid by the method of Friedemann, Cotonio, and Shaffer as modified by Wendel (8). All chemical measurements are expressed in milligrams per 100 grams muscle. The hexosephosphate hexose figures only are tabulated; the usual agreement between P calculated (from hexose figures) and P found was observed throughout. In 63 observations covering a range of 7 to 29 mgm. of hexosephosphate P per 100 grams muscle, the average P value calculated from hexose figures was 16.6 as compared to the average of 18.0 mgm. per cent from directly determined P values.

Stimulation. The rates chosen were 10 and 60 per minute, for it was soon found that these are about the limits at which one can elicit 60 contractions and avoid on the one hand contracture and fatigue, and on the other an undue difference in the length of time necessary to produce 60 twitches. The stimuli were condenser discharges timed by a neon tube circuit, and were always indirect, descending, and supramaximal.

Tension record. The tension lever consisted of a length of clock spring with a writing lever attached at right angles, and allowed the muscle to shorten about 0.7 mm. per 100 grams tension. After each experiment a direct calibration by weights suspended over a pulley was run onto the record. For single shocks tension was summated and multiplied by the length of the muscle in centimeters, and for tetani the tension-length-time product was obtained, but no correction for the arrangement of fibers in the gastrocnemius was made in either case. In order to compare tension with the chemical data (which are given per unit of muscle weight) the tension product was divided by the weight of the muscle in grams.

Total tension in the tables is expressed in kilograms per gram muscle, in which case the isometric coefficient for lactic acid ($K_{m(L)}$) is given by the expression

$$\frac{\text{total tension per gm. muscle (as tabulated)}}{\text{mgm. per cent increase in lactic acid}} \times 100.$$

Substituting values from experiment 1, table 1, $K_{m(L)} = \frac{7}{4}\frac{5}{6} \times 100 = 163.10^6$. Isometric coefficients are not tabulated, but may be calculated in this way from data given in the tables.

Effect of rate of stimulation on lactic acid and hexosephosphate. In experiments 1 to 6, table 1, one of a pair of muscles was stimulated at a rate of

10 or 60 per minute, while its mate was fixed without stimulation for determination of basal values for lactic acid and hexosephosphate. In experiments 7a to 10a and 7b to 10b, table 1, the basal values could not be determined, since one of a pair of matched muscles was stimulated at a rate of 10 and the other at a rate of 60 per minute.

TABLE 1

Effect of sixty contractions against tension lever on lactic acid and hexosephosphate

Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilograms per gram muscle.

EXPERIMENT NO.	RATE OF STIMULATION (PER MINUTE)	TOTAL TENSION	RESTING		STIMULATED	
			Lactic acid	Hexose-phosphate	Lactic acid	Hexose-phosphate
1	10	75	34	68	80	95
2	10	49	22	55	44	83
3	10	61	22	44	64	84
Average		*62*	*26*	*56*	*63*	*87*
Less basal					*26*	*56*
Increase					37	31
4	60	57	25	50	51	122
5	60	42	17	52	53	101
6	60	78	18	39	71	113
Average		*59*	*20*	*47*	*58*	*112*
Less basal					*20*	*47*
Increase					38	65
7a	10	46			47	67
8a	10	62			83	92
9a	10	62			61	82
10a	10	67			60	61
Average		*59*			*63*	*75*
Less basal*					*23*	*51*
Increase					40	24
7b	60	45			51	94
8b	60	49			64	110
9b	60	71			83	98
10b	60	72			70	98
Average		*59*	*23*	*51*	*67*	*100*
Less basal*					*23*	*51*
Increase					44	49

* Average basal values from experiments 1 to 6.

On comparing the effect of different rates of stimulation on lactic acid production one finds that in experiments 1 to 3, the "slow" series, with an average tension of 62 kgm. per gram muscle, 37 mgm. per cent of lactic

acid was formed, while in experiments 4 to 6, the "fast" series, with a nearly equal amount of tension, 38 mgm. per cent of lactic acid was produced. Similarly, in experiments 7 to 10, a and b, with equal amounts of tension, levels of 63 and 67 mgm. per cent of lactic acid were attained, which, after deduction of the average basal value, indicate lactic acid production of 40 and 44 mgm. per cent, respectively. It may be seen that stimulation at different rates, resulting in work of equal magnitude without fatigue, has little, if any effect on the amount of lactic acid formed as a result of contraction.

While the rate of stimulation produces no change in the amount of lactic acid formed, it has a marked effect on hexosephosphate formation. Muscles contracting at a rate of 60 per minute showed an average hexosephosphate increase which was twice that found in muscles producing a nearly equal amount of tension at the rate of 10 per minute (compare experiments 1 to 3 and 7a to 10a with experiments 4 to 6 and 7b to 10b, table 1).

In these experiments the muscles were fixed immediately after cessation of stimulation, but since 6 minutes were required for 60 contractions in the slow and only one minute in the fast series, the objection could be made that the difference in hexosephosphate observed was due to the greater length of time available for disappearance of the ester in the slow as compared to the fast series. Such a supposition could be entertained only if hexosephosphate disappeared as rapidly as 34 mgm. per cent in five minutes, but in previous experiments (1) such rates of disappearance were not found either aerobically or anaerobically. As a matter of fact, in muscle kept anaerobically there occurred a slight rise in hexosephosphate content in the first 30 minutes after stimulation. Nevertheless, it seemed advisable to test this point once more. Consequently another series of experiments was performed, identical with the "slow" and "fast" series in table 1, with the exception that the muscles stimulated at 60 per minute were allowed to remain in N_2 for 5 minutes after stimulation, so that the time elapsing between the beginning of stimulation and fixation was the same in the two series. The results (table 2) were identical in kind with those in table 1. For similar amounts of tension (64 and 68 kgm. per gm. muscle) similar levels of lactic acid (74 and 73 mgm. per cent) were attained; yet the rapidly stimulated muscles contained on an average 31 mgm. per cent more hexosephosphate than did those stimulated slowly.

Comparison of tetani and single contractions. It seemed of interest to determine whether tetanic stimulation would have a still greater effect on hexosephosphate increase than single shock stimulation at the rate of 60 per minute. Since the two forms of contraction cannot be compared on the basis of tension (because the tension-time product is determined in tetani and not in twitches), it was necessary to make the comparison on the basis of some chemical change related to tension such as phospho-

creatine breakdown or lactic acid formation. Tetani of 10 seconds' duration were chosen because such tetani produced about the same amount of lactic acid as did 60 twitches.

In 18 such experiments the average amount of lactic acid formed was 39, as compared to 46 and 43 mgm. per cent in 60 single contractions at 60 and 10 per minute, respectively (see table 3). Per milligram lactic acid formed

TABLE 2

Influence of rate of stimulation on lactic acid and hexosephosphate in muscles with resting periods of equal length

A. Stimulated 60 times at 10 per minute and fixed immediately.

B. Stimulated 60 times at 60 per minute and fixed after 5 minutes in nitrogen.

Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilograms per gram muscle.

EXPERIMENT NO.	TOTAL TENSION		LACTIC ACID		HEXOSEPHOSPHATE	
	A	B	A	B	A	B
11	83	86	81	87	69	112
12	53	64	86	79	77	104
13	55	54	55	53	54	79
Average..	64	68	74	73	67	98

TABLE 3

Relation of lactic acid and hexosephosphate changes during contraction

TYPE OF STIMULATION	10 SECONDS TETANUS	60 SINGLE SHOCKS	
		60 per minute	10 per minute
Number of experiments	18	15	10
Lactic acid formed, mgm. per cent	39	46	43
Hexosephosphate formed, mgm. per cent hexose	63	54	25
Sum of lactic acid and hexosephosphate	102	100	68
Mgm. hexosephosphate per mgm. lactic acid	1.6	1.2	0.6
Mgm. carbohydrate* broken down per mgm. lactic acid formed	2.6	2.2	1.6

* Sum of lactic acid and hexosephosphate formed.

the increase in hexosephosphate was greater in the tetani than in the single contractions at 60 per minute, showing that the full effect of rate had not been reached with the latter form of stimulation.

Since hexosephosphate is formed from glycogen and accounts, together with lactic acid, for most of the glycogen disappearing as the result of contraction (10, 11), it seemed permissible to interpret the above results in terms of glycogen. In table 3, the total carbohydrate (or glycogen) broken down per unit of lactic acid formed has been calculated. The figures

show that the largest amount of glycogen is broken down in tetani, less in single contractions at 60 per minute, and still less in 60 contractions at 10 per minute.[1]

Relation of length of tetanus and degree of isometrism to after-formation of lactic acid. The comparison of tetani and single contractions on the basis of lactic acid production, as was done in table 3, would not be correct if the conditions of the experiments permitted an appreciable amount of the lactic acid formed to appear after the contraction. Delayed formation of lactic acid in the case of muscles stimulated at 60 per minute has already been ruled out by the experiments in table 2, where a resting period of 5

TABLE 4

Relation of delayed formation of lactic acid to length of tetanus and degree of isometrism

Matched muscles, (1) fixed 4 to 8 seconds after stimulation; (2) fixed 3 minutes after stimulation. Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. Total tension is given in kilogram-seconds per gram muscle. All figures are averages.

	GROUP					
	A		B		C	
Number of experiments	5		3		4	
Length of tetanus (sec.)	3–6		10		10	
Shortening per 100 gm. tension (mm.)	0.7		0.7		0.04	
Muscle	1	2	1	2	1	2
Total tension	10	10	21	21	44	39
Lactic acid	35	41	62	66	65	71
Hexosephosphate	76*	80*	126**	120**	136**	145**
Lactic acid increase‡	12	18	39	43	42	48
Lactic acid formed per unit of tension	1.2	1.8	1.86	2.05	0.95	1.23
After formation in per cent of total		33		9		23

* Four experiments averaged.

** Single experiment.

‡ See table 1 for basal values.

minutes after stimulation did not result in additional formation of lactic acid. In 3 experiments (group B, table 4) matched muscles were tetanized for 10 seconds against the tension lever used in the previous experiments, one muscle being fixed within 6 seconds while the other was allowed to remain in N_2 for 3 minutes after stimulation. The average $\frac{\text{lactic acid}}{\text{tension}}$ ratios in these experiments indicate a delayed formation of lactic acid amounting to but 9 per cent of the total.

[1] These differences need not necessarily be reflected in heat measurements because the heat liberated in the reactions glycogen → hexosephosphate is small as compared to the reactions glycogen → lactic acid.

In view of the slight after-formation observed under these conditions, an attempt was made to define conditions under which it occurred. Muscles were subjected to short (3 to 6 second) tetani against the same spring, and in this case a delayed formation of 33 per cent of the total (group A) was observed. This suggested that with similar initial tensions the after-formation of lactic acid becomes greater as the time allowed for completion of the chemical changes leading to lactic acid becomes shorter. In such a case it should also be possible to increase after-formation of lactic acid in a long tetanus by allowing the muscle to produce more tension. A more nearly isometric lever and optical registration was used in the experiments in group C, allowing the muscle to shorten 0.04 mm. per 100 gram tension as compared to 0.7 mm. per 100 gram tension for the lever used in groups A and B. A definite delayed lactic acid formation was found amounting to 23 per cent of the total amount of lactic acid formed. These experiments support another observation which relates delayed lactic acid formation to tension.[2]

Relation of lactic acid and hexosephosphate to work. It was noted that, in general, the increase in lactic acid seemed to be roughly proportional to the tension, while hexosephosphate was not so related. Because the nature of the experiments prevented the determination of basal values for each experiment, averages only could be used to demonstrate this observation. The experiments were tabulated in an ascending order of work, and averages for tension, lactic acid, and hexosephosphate were calculated for the first and second half of each group of experiments. From the average levels the basal values (table 1) were deducted to find increases in lactic acid and hexosephosphate. Columns 2 and 3 in table 5 represent the average figures so obtained.

In column 1 are presented data from experiments designed to produce contraction with the least possible amount of work. The muscles were stimulated without being attached to the tension lever so that their work consisted mainly in overcoming viscosity and gravity. While only a small amount of lactic acid (8 and 9 mgm. per cent) was formed, there was a considerable increase in hexosephosphate in both single shock and tetanic experiments (31 and 24 mgm. per cent, respectively). The much greater increase in hexosephosphate than in lactic acid indicates that hexosephosphate formation may be a process not entirely associated with work performed by a muscle. This suggestion is supported by experiments in which epinephrine was allowed to act on isolated frog muscle (6). Under

[2] Meyerhof and Schulz failed to observe delayed lactic acid formation in one series of experiments (9), while subsequently (3) they succeeded in demonstrating this phenomenon. These authors ascribed the failure to observe delayed lactic acid formation to the fact that much lower tensions were developed in the former than in the latter series of experiments.

anaerobic conditions epinephrine caused the formation of about 5 mgm. of hexosephosphate per milligram of lactic acid, a ratio similar to that produced by contractions without load. In the latter case some work is undoubtedly performed, and some phosphocreatine might be expected to be broken down, while in the former case epinephrine did not cause a decrease in phosphocreatine.

TABLE 5

Relation of lactic acid and hexosephosphate to work

Column 1, from experiments without load. *Column 2*, first half, and *Column 3*, second half of series of tension lever experiments. See text. Lactic acid and hexosephosphate (as hexose) are given in milligrams per 100 gram muscle. All figures are averages.

	60 CONTRACTIONS IN 1 MINUTE		
Column number	1	2	3
Number of experiments averaged	3	8	8
Total tension (kgm. per gm. muscle)		54	76
Lactic acid increase	8	39	54
Hexosephosphate increase	31	52	55
Lactic acid formed per unit of tension		0.72	0.71
Mgm. hexosephosphate formed per mgm. increase in lactic acid	3.9	1.3	1.0
Mgm. carbohydrate* broken down per mgm. lactic acid formed	4.9	2.3	2.0
	10 SECOND TETANI		
Column number	1	2	3
Number of experiments averaged	3	9	9
Total tension (kgm.-sec. per gm. muscle)		20	31
Lactic acid increase	9	33	46
Hexosephosphate increase	24	59	66
Lactic acid formed per unit of tension		1.6	1.5
Mgm. hexosephosphate formed per mgm. increase in lactic acid	2.7	1.8	1.4
Mgm. carbohydrate* broken down per mgm. lactic acid formed	3.7	2.8	2.4

* Sum of lactic acid and hexosephosphate formed.

The ratios $\frac{\text{lactic acid}}{\text{tension}}$ in columns 2 and 3 in table 5 indicate that lactic acid formed was proportional to tension within the range of these experiments. Such a proportionality does not exist in the case of hexosephosphate. Since no measurements of tension could be made in the case of contractions without load the $\frac{\text{hexosephosphate}}{\text{lactic acid}}$ ratio has been calculated. This ratio shows that in both single contractions and tetani, the greatest

increase in hexosephosphate per milligram of lactic acid occurred when the work done was very slight (contractions without load); that with intermediate amounts of tension less hexosephosphate was formed per milligram of lactic acid; and that with large amounts of tension still less was formed. In terms of glycogen this means that the amount broken down per milligram of lactic acid (or per unit of tension) decreases as the work performed increases.

The expenditure of glycogen during contraction depends not only on the work performed by a muscle but also on the rate of stimulation. Both these factors must be taken into account in order to determine the conditions under which a muscle can perform the greatest amount of work with the least expenditure of glycogen. The optimal conditions have not as yet been determined, but the experiments show that single contractions at a slow rate, with each contraction producing a great deal of tension, cause the smallest breakdown of glycogen per unit of work. More glycogen per unit of work is expended in the same number of contractions at a more rapid rate producing the same amount of work, or contractions at the slow rate producing less work. Still more glycogen is broken down in tetani, while relatively the greatest expenditure of glycogen occurs in contractions without load.

SUMMARY

1. Sixty single contractions of isolated frog gastrocnemii against a tension lever led to the production of equal amounts of work and lactic acid when the rate of stimulation was either 10 or 60 per minute. In the former case, however, the increase in hexosemonophosphate was about half that in the latter case. A single ten second tetanus produced approximately the same amount of lactic acid, and still more hexosephosphate than did the contractions at 60 per minute. Since hexosephosphate is formed from glycogen, it follows that a greater breakdown of glycogen occurs in rapidly than in slowly stimulated muscles performing equal amounts of work.

2. In 60 single contractions or a ten second tetanus of an unloaded muscle about half as much hexosephosphate and one-fifth as much lactic acid was formed as in similar contractions of a muscle against a tension spring.

3. In contrast to lactic acid, hexosephosphate was not directly related to the work performed. Per milligram of lactic acid formed the increase in hexosephosphate was greatest in contractions without load and became smaller for increasing amounts of work. It may be concluded that, considering the effects of both rate of stimulation and work, a muscle can produce the greatest amount of work with the least expenditure of glycogen if it is producing maximal tension at a slow rate of stimulation.

4. Significant delayed formation of lactic acid was not observed after 10

second tetani against a spring permitting considerable shortening of the muscle. After-formation was observed, however, following short tetani against a weak spring, and following 10 second tetani against a stiff tension lever. In no case was there a significant delayed formation of hexose-phosphate.

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THE ORIGIN AND SIGNIFICANCE OF NEUTRAL CHLORIDE IN THE SECRETIONS OF THE STOMACH AND DUODENUM

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Received for publication January 24, 1935

During the past three years a large number of experiments have been performed in this laboratory in which standard hydrochloric acid solutions have either been introduced into pouches of various parts of the stomach and duodenum or have been mixed with the secretions from these parts. An analysis of these data has thrown considerable light on the origin and significance of the neutral chloride present in gastric contents. The present communication presents an analysis of these experiments.

MATERIAL. The present analysis is based on 95 experiments comprising 12 on whole stomach pouches; 20 on isolated pyloric pouches; 19 on isolated duodenal pouches; 21 on the secretions from the intact duodenum obtained after stimulation with acid; 12 in dogs with gastroduodenostomy; 11 in dogs with gastrojejunostomy just below the ligament of Trietz.

METHODS AND CALCULATIONS. The methods and calculations have been described in detail in previous publications and will only be briefly outlined here. The general procedure was to introduce a standard hydrochloric acid solution (approximately tenth normal) containing phenol red, into the pouch to be studied and to allow it to remain for one half-hour; at the end of one half-hour the pouch was emptied as completely as possible and fresh acid solution introduced, at the end of the second half-hour period the pouch was again refilled. From three to four half-hour samples were usually obtained consecutively. In the whole stomach pouches and in the stomachs with gastroduodenostomy and gastrojejunostomy, the stomach was not stimulated with histamine and only those experiments in which there was no evidence of secretion of hydrochloric acid by the stomach have been considered in the present analysis. In the experiments with duodenal secretions obtained from the intact duodenum, the procedure was to stimulate the duodenum by introducing the hydrochloric acid-phenol red solution through a duodenal tube; when a copious flow of secretion occurred the secretions were removed and mixed with the same

TABLE 1

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
349	98	15	6	9	40	450	
	98	15	9	6	60	300	
351	93	30	5	25	17	357	
	96	26	9	17	35	425	
	96	24	13	11	54	275	
351	96	17	3	14	18	350	Whole stomach pouches
	96	19	7	12	37	300	
351	93	28	7	21	25	300	
	89	46	11	35	24	319	
351	94	21	6	15	29	250	
	89	39	3	36	8	328	
	88	66	23	43	35	358	
Average					32	334	
348	79	123	47	76	38	362	
	84	81	13	68	16	425	
	85	79	13	66	16	440	
	83	92	29	63	32	370	
	81	99	24	75	24	395	
	81	98	28	70	29	370	
	78	139	40	99	29	450	
	75	134	30	104	22	416	
	71	141	23	118	16	407	
	84	61	9	52	15	325	Pyloric pouches
	92	41	13	28	32	350	
	91	67	40	27	60	300	
	86	60	4	56	7	400	
	89	48	13	35	27	318	
	61	211	71	140	34	359	
	70	143	38	105	27	350	
	70	143	34	109	24	363	
	67	162	43	119	27	361	
	74	121	28	93	23	358	
	80	126	58	68	46	340	
Average					27	373	
Average of 32 experiments					29	358	

TABLE 2

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
348	55	172	45	127	26	282	
	65	152	61	91	40	260	
	65	162	82	80	51	230	
	62	187	84	103	45	271	
	69	170	87	83	51	268	
	63	188	94	94	50	254	
	48	206	37	169	18	325	
	61	186	63	123	34	315	
	58	191	63	128	33	305	
	54	166	4	162	2	352	Isolated duodenal pouches
	58	177	39	138	22	329	
	65	165	43	122	26	349	
	60	165	33	132	20	330	
	57	188	52	136	28	316	
	50	214	32	182	15	364	
	53	211	47	164	22	345	
	56	229	75	154	33	327	
	58	231	91	140	39	333	
	46	302	120	182	40	336	
Average					31	310	
350	81	103	39	64	38	336	
	79	89	8	81	9	386	
	82	93	25	68	27	377	
	85	75	22	53	29	353	
348	63	201	90	111	45	300	
	61	188	76	112	40	288	
	63	195	86	109	44	295	Duodenal secretions from intact duodenum
347	82	96	38	58	40	322	
	82	100	53	47	53	261	
	88	47	17	30	36	250	
	82	84	26	58	31	322	
347	70	125	38	87	30	290	
	71	125	23	102	18	352	
	77	113	41	72	36	313	

TABLE 2—*Concluded*

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE, FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
352	82	54	4	50	7	278	Duodenal secretions from intact duodenum —*Concluded*
	86	50	11	39	22	278	
	89	44	21	23	48	210	
	86	46	13	33	28	236	
348	76	95	17	78	18	325	
	79	89	19	70	21	334	
	79	80	9	71	11	338	
Average					30	307	
Average of 40 experiments					30	308	

strength acid-phenol red solution that had been introduced into the duodenum.

The following analyses were made on the acid-phenol red solution containing the secretions: 1. The per cent of phenol red present after removal of interfering substances with 10 per cent sodium tungstate and two-thirds normal sulfuric acid. 2. Total and neutral chloride after careful ashing of the dried aliquot sample.

The calculations were as follows: The decrease in the per cent of phenol red in the mixture of acid and secretion showed how much secretion was present in 100 cc. of the mixture. When the acid chloride concentration of the original acid solution was multiplied by the per cent of phenol red in the sample, the result gave the original acid solution corrected for dilution. The total chloride concentration of the sample removed from the pouch was always above that of the original acid solution corrected for dilution, and the difference represented the chloride content of the secretions which were mixed with the acid-phenol red solution. When the extra chloride was divided by the amount of secretion in the sample (obtained from the decrease in the per cent of phenol red) the result gave the chloride concentration of the secretions. The chloride present in the secretions was neutral chloride. The acid chloride concentration of the sample removed from the pouch was always below that of the original acid solution corrected for dilution, and the difference represented hydrochloric acid that had been neutralized by the secretion of the pouch; the chloride of this neutralized acid was, of course, present with the neutral chloride fraction.

The total neutral chloride in the sample removed from the pouch could thus be divided into two fractions: a, the neutral chloride which was a constituent of the secretion, and b, neutral chloride which resulted from neutralization of hydrochloric acid by the secretions. From these data

it was possible to calculate the per cent of the total neutral chloride which resulted from neutralization of hydrochloric acid by the secretions of the pouch.

TABLE 3

ACID SOLUTION USED, MGM. ACID CHLORIDE PER 100 CC.	P.S.P., PER CENT	TOTAL NEUTRAL CHLORIDE, MGM. PER 100 CC.	NEUTRAL CHLORIDE FROM NEUTRALIZED ACID, MGM. PER 100 CC.	EXTRA NEUTRAL CHLORIDE, MGM. PER 100 CC.	PER CENT NEUTRAL CHLORIDE FROM NEUTRALIZED ACID	CHLORIDE CONCENTRATION OF SECRETION, MGM. PER 100 CC.	EXPERIMENT
348	75	134	55	79	41	316	
	78	119	51	68	43	309	
	79	116	46	70	40	333	
351	78	94	17	77	18	350	
	76	138	57	81	41	337	
	77	122	44	78	36	339	Gastroduodenostomy
348	68	111	12	99	11	310	
	67	118	16	102	14	310	
	69	103	9	104	9	303	
351	65	127	25	102	20	291	
	69	116	21	95	18	307	
	73	111	34	77	31	286	
Average					27	316	
349	74	114	30	84	26	323	
	77	109	42	67	39	292	
	81	100	40	60	40	316	
349	78	87	17	70	20	318	
	77	111	40	71	36	309	
	82	102	46	56	45	311	Gastrojejunostomy
351	71	147	49	98	33	338	
	81	89	29	60	33	316	
351	61	142	5	137	4	351	
	72	98	8	90	8	322	
	81	80	12	68	15	358	
Average					27	323	
Average of 23 experiments					27	319	

RESULTS. The results are given in tables 1, 2, and 3. In column 7 of each table is shown the chloride concentration of the secretion under consideration. It is seen that the non-acid secretions of the whole stomach and the secretions of the pylorus (table 1), the mixed duodenal secretions (bile, succus entericus and pancreatic juice) (table 2), and the combined

duodenal and non-acid gastric secretions (table 3) have approximately the same chloride concentration, the average values for each secretion ranging from 373 to 307 mgm. of neutral chloride per 100 cc. The slightly higher value for the chloride concentration of the non-acid secretion of the stomach has again been confirmed in recent unpublished experiments. The average value for the 95 experiments shown in tables 1, 2 and 3 is 327 mgm. per 100 cc.

The per cent of the total neutral chloride which is the result of neutralization of hydrochloric acid by the various secretions, is shown in column 6 of each table. The average values for the individual groups of experiments range from 27 to 32 per cent, and show relatively little differences between the secretions of the various parts of the stomach and of the duodenum. The average value for the 95 experiments is 29 per cent.

Discussion. Since all of the secretions investigated may at times be found in the stomach, it is quite obvious that it is a definite error to consider that all of the neutral chloride present in the gastric contents represents neutralized hydrochloric acid; on the average, approximately only one-third of the neutral chloride actually represents neutralized hydrochloric acid, the remaining two-thirds having entered the stomach as a constituent of the various non-acid secretions.

The chloride concentration of the non-acid secretions of the stomach and the mixed duodenal secretions is very close to the blood chloride level. Previous studies (1, 2, 3, 4), have shown that the alkalinity of these various secretions is approximately the same, the average value being 0.04 normal.

The values for the neutral chloride concentration and for the alkalinity of the various non-acid secretions which may at times enter the stomach are so similar that in any given case it would be practically impossible to tell whether they were of intragastric or duodenal origin.

SUMMARY

In a series of 95 experiments it was found that the neutral chloride concentrations of the non-acid secretions of the stomach and the mixed duodenal secretions were approximately the same, the average values ranging from 358 to 307 mgm. per 100 cc.

When the non-acid secretions of the stomach or the duodenal secretions are mixed with tenth normal hydrochloric acid, only about one-third of the neutral chloride fraction represents neutralized hydrochloric acid.

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STUDIES ON THE RATE OF DISAPPEARANCE AND FATE OF MARE GONADOTROPIC HORMONE FOLLOWING INTRAVENOUS INJECTION

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Received for publication January 23, 1935

Gonad-stimulating hormones are known to circulate in quantity during pregnancy in the blood streams of two widely separated mammalian groups: namely, man and the higher apes, and the family Equidae (horse, ass and zebra). The hormone of the first group has been investigated widely as prolan; that of the second group has been dealt with, as regards the conditions of its appearance, its distribution in the mare, and its properties, in a series of papers cited by Catchpole and Lyons (1934). Prolan and the mare gonadotropic hormone show marked physiological differences which have been enumerated in the above reference. The present paper concerns a further property of the mare serum hormone in which its difference from prolan is emphasized. It is well known that prolan passes the kidney with ease; its concentration in the urine of pregnant women agrees with its concentration in the blood stream itself. Parkes and White (1933) injected prolan into rabbits intravenously and studied in a roughly quantitative manner the passage of hormone into the urine. They reported that one-third or less of the injected hormone was excreted in 9 hours, and that the blood renal threshold for prolan, if any, was low. The fate of the remaining two-thirds was undetermined in their experiments. There might have been some destruction in the blood, and also the excretion might have continued after 9 hours. Their conclusion that the failure of the pregnant rabbit to excrete prolan was due to a lack of hormone in the blood was justified only as far as hormones of the prolan type are concerned. Hormones of the pregnant mare type do not pass through the kidney. The first indication of this was a practical failure to recover gonadotropic hormone from the urines of pregnant mares, even at periods when the concentration of hormone in the bloods of the animals was very high. Rarely, small amounts of hormone do appear in the urine. The figures of Zondek (1931) probably refer to one of these exceptional occasions and are not of general significance. A number of qualitative experiments by various authors bear out this difference between prolan and the mare gonadotropic hormone. Evans (1933) found that prolan was recover-

able from urines of rats injected with this substance, and Ehrhardt (1930) found that a non-pregnant woman excreted prolan transfused from a pregnant one. Evans interestingly observed that a rhesus monkey excreted hormone after the injection of a preparation from hypophysis itself (of the pig). But both rats and monkeys failed to excrete active urines following injection of mare gonadotropic hormone. The relative difficulty of excretion of mare hormone by the kidney raises the question of the fate of the hormone in the animal body. This paper is concerned with the rate of disappearance and the fate of pregnant mare hormone following intravenous injection of rabbits and gelding.

EXPERIMENTAL. Massive doses, as expressed in rat units, of potent pregnant mare blood sera were injected into the blood streams of the experimental animals. After a short interval of time, to allow thorough mixing throughout the circulatory system, an initial blood sample was withdrawn, and thereafter samples withdrawn at fixed intervals of time. These samples were assayed for their gonadotropic hormonal content by a method similar to that proposed by Cole et al. (1932). The rat unitage was found by giving a series of dilutions of the order of 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ cc., etc. In the gelding, where plenty of serum was available, a rather more accurate estimate was made by further grading the dose in the vicinity of the rat unit level. After the first few assays, the approximate unit could be roughly predicted, and many of the initial assays eliminated.

Disappearance and fate of mare gonadotropic hormone in the rabbit. Young New Zealand white female intact and castrated rabbits were used. It was soon found that ovariectomy produced no difference in the picture of hormone disappearance showing that the gonads do not contribute appreciably to the rapid destruction of hormone which occurs. The intact animals responded by ovulation to the injection of hormone, but the amount of hormone, if any, used up to effect this must be an insignificant portion of the total amount injected. We know of no other data on actual utilization of gonadotropic hormones by the gonads.

Most of the animals were injected in a left ear vein with 30 cc. of pregnant mare serum containing 100 rat units per cc., a total of 3,000 rat units. Usually, the only untoward effect on the animal was a transient increase in the respiration, but in two cases we encountered immediate death from shock. Fifteen to 30 minutes after the injection a 1 cc. sample of blood was withdrawn from the other ear. This amount was sufficient since the initial concentration reached by the hormone in the serum of the rabbit was as high as 40 rat units per cc.[1] The size of the samples taken was kept to a minimum to avoid the removal of a significant amount of hormone by this route. Later, when the concentration of hormone had fallen considerably, it was necessary to obtain larger samples. Sampling was done at 24 hour intervals, and was continued till the 120th or 150th hour, when

[1] This figure agrees with estimates based on the blood volume of these rabbits.

the amounts of hormone became too small to estimate. In some preliminary experiments only 1,000 rat units were injected, but the smaller concentrations of hormone made estimation less accurate. The curves relating hormone disappearance to time, and the logarithm of hormonal concentration to time, for one group appear in figure 1. In two cases the experiments were conducted in metabolism cages. The urine collected up to 144 hours after injection was precipitated in acetone and the resultant

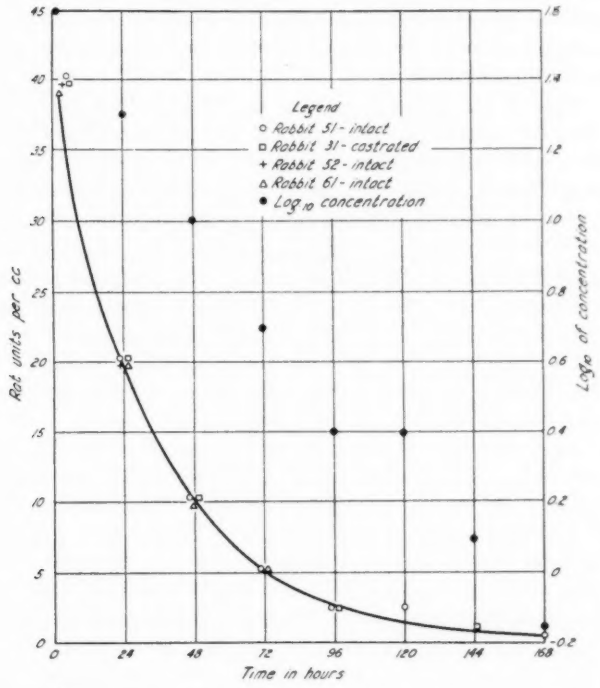

Fig. 1. Disappearance of mare gonadotropic hormone from blood of rabbits following intravenous injection. Each of the four rabbits received an intravenous injection of 3,000 R.U.

powder extracted for gonad-stimulating hormone. Doses equivalent to $\frac{1}{10}$ of the total urine given to immature rats elicited no response, although over the time that the urine was collected practically 3,000 rat units had disappeared from the body. Nor was hormone detected in feces, but all preparations from this source were almost too toxic to assay.

Two animals were sacrificed at 24 hours, following the usual injection of 3,000 rat units. At this time, approximately 1,500 rat units remained in the blood serum at a concentration of 20 rat units per cc. and 1,500 rat

units had disappeared. The uterus, spleen, lungs, kidneys, and liver of each of these animals were removed and each tissue separately extracted for gonad-stimulating hormone by the method of Catchpole and Lyons (1934). Injection of 1 gram equivalents of these glands and tissues in no case evoked a response in the test animals, proving that there was no appreciable storage of hormone at any of these sites.

Disappearance of mare gonadotropic hormone from the gelding. It was decided to continue the line of experimentation followed above by studying the course of pregnant mare serum hormone destruction in the horse itself, following its introduction into the blood stream. The proteins introduced in this case were more nearly homologous with the blood proteins of the recipient than in the rabbit experiments. Further we had a series of figures upon the rate of disappearance in the pregnant mare with which the results might profitably be compared. In the pregnant mare, after maintaining a plateau of high hormonal concentration, the hormone level starts to drop at about the 100th day of gestation (Cole and Saunders, 1935). Should the hormonal production in the mare suddenly cease, then the condition in the mare will be the same as if a certain amount of hormone had been introduced into the blood and left to disappear, and the rate of this disappearance should parallel that of an injected animal.

We used a castrated horse (gelding) weighing 1,100 pounds. About 3 liters of mare serum, containing about 90,000 rat units of hormone, were introduced into the left jugular vein with a large needle. The animal exhibited rather alarming systemic effects for some time after the injection, with marked increase in the respiration, and intestinal movements with defecation, reminiscent of anaphylactic shock. There was an edema of the breast and stiffness in the joints for about two weeks. After the first day the appetite was normal. A blood sample was withdrawn from the right jugular vein after the lapse of 1 hour. Samples were taken at 24 hour intervals for 1 week, then at 2 or 3 day intervals, and finally at 6 day intervals. Sampling was continued for 27 days in all, by which time the level of hormone had fallen so low that without extractive measures, an inconveniently large amount of serum had to be injected into rats to elicit a response.

The initial concentration of hormone in the blood serum produced by the injection of some 90,000 rat units into a horse of this size was 5 rat units per cc., a figure sufficiently close to that expected by calculation from the blood volume of the horse. It may be compared with the figure of 50 to 100 rat units per cc. reached in the pregnant mare. Figure 2 shows the curve of hormonal concentration plotted against time for the injected gelding.

Discussion. In spite of the lack of appreciable urinary excretion, mare gonadotropic hormone introduced into the circulation disappears from it relatively rapidly, both in the intact and castrated animal. The curves

showing the relation between concentration and time are by no means simple in form. Cole and Saunders (1935) showed that hormone disappearance from the pregnant mare followed a logarithmic curve, with the concentration being halved every 8 to 10 days. In the gelding, there is a fall in hormonal concentration to $\frac{1}{2}$ in the first 72 hours after injection, but thereafter the rate of destruction decreases and during the next 20 days there is a rather regular fall in hormone to $\frac{1}{2}$ every 6 days. There is thus a definitely greater destruction rate in the gelding than in the mare, a difference which may be a real one, or more probably due to a persistence of

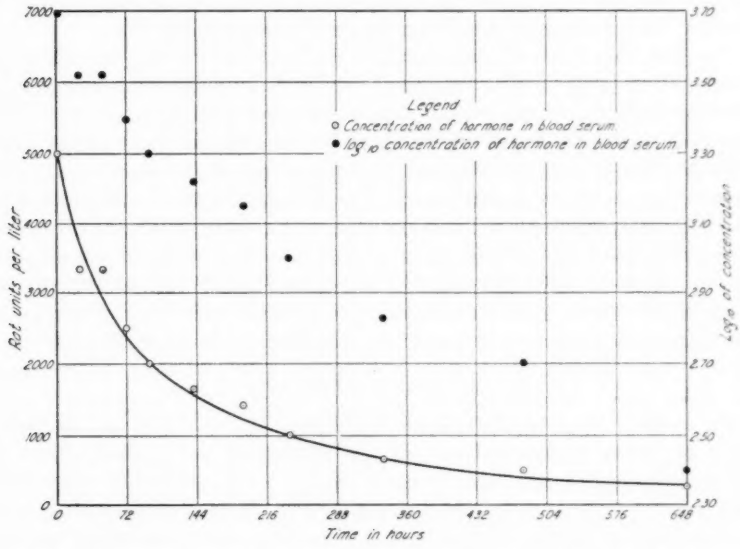

Fig. 2. Disappearance of mare gonadotropic hormone from blood of a gelding following intravenous injection.

hormonal production in the mare, but at a diminishing rate, even over the period when hormone is disappearing absolutely from the body of the mare.

In the rabbit the exact conditions of destruction are harder to define, as the absolute rate of decrease of hormone is very much greater, its concentration in the blood being reduced to $\frac{1}{2}$ every 26 hours. It is possible that, as in the gelding, the initial fall in hormonal concentration is greater than in subsequent decreases, because an inspection of the earlier part of the rabbit curve would indicate a faster disappearance of hormone than observations taken after several days actually show. Assuming the interpretation of a somewhat greater initial destruction of hormone in both rabbit and gelding, the rabbit values after 48 hours and the gelding values after 72 hours give approximate linear logarithmic curves; however, from

the present results we hesitate to dogmatize as to the exact form of these concentration curves.

These experiments confirm the differences already noticed between pregnant mare hormone and prolan. Whereas the latter is rapidly excreted in the urine, the former remains in the blood stream until it is destroyed. This property of the hormone may render it of particular importance therapeutically; the persistence of the hormone for some time in the blood stream allows its action on the gonads to approximate more closely to a continuous one than does that of the readily excretable prolan. This is undoubtedly an explanation of the finding of Cole et al. (1932) that single doses of the hormone are as efficacious as split doses in the rat.

The fact that the body can destroy this hormone throws a new light on hormonal relationships in the mare itself. It is difficult to escape the conclusion that destruction occurs from the first, and probably at much the same rate as we have demonstrated for the gelding. This would indicate that the production of hormone in the pregnant mare is much larger than a study of hormonal concentrations at any one time would indicate. The present experiments indicate that the animal body is capable of metabolising and destroying this hormone promptly, at a rate depending roughly on the concentration of hormone in the blood stream, and on the animal species used. Further, they indicate that the fetus and its membranes are not concerned in the destruction of the hormone in the pregnant mare.

SUMMARY AND CONCLUSIONS

Data are given showing the rate of disappearance of mare gonadotropic hormone from the blood stream of the rabbit and the gelding following administration of large doses of the hormone. The hormonal concentration was reduced approximately one-half every 26 hours in the rabbit and one-half every 6 days in the gelding. The latter rate is definitely greater than in the pregnant mare and indicates that, in the mare, hormone is being produced coincidentally with its destruction. There is no evidence either that the hormone is excreted in the urine or feces of the rabbit or that it is stored in the uterus, spleen, lungs, kidneys, or liver. Thus it is concluded that the hormone is destroyed within the animal body. As the rates of disappearance are similar in castrated and non-castrated rabbits it is improbable that the gonads play a significant rôle in its destruction.

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RESERVE STORE OF HEMOGLOBIN PRODUCING SUBSTANCES IN GROWING DOGS AS INFLUENCED BY DIET

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Received for publication February 1, 1935

Early in our anemia work we suspected a reserve store of materials out of which hemoglobin could be produced but this could not be demonstrated by the short anemia experiments which were first used (4). When the long continued anemia due to blood loss in dogs was substituted for the short anemia experiments we soon had evidence of a reserve store out of which the dog could fabricate much hemoglobin. Moreover when a favorable diet such as liver was fed during a 2-week period we observed much new hemoglobin production in the second week of liver feeding but almost as much in the 2 control weeks of bread following the liver diet. If the dog produced 100 grams new hemoglobin as a result of the liver diet we observed that about 40 grams appeared in the after control period of 2 weeks —a "carry over" due to *storage* of hemoglobin building materials in the body which had to be exhausted before the dog was back to its base line level of hemoglobin production due to standard bread feeding. If the favorable diet regimen continued longer could the body store greater amounts of this hemoglobin producing material? The tabulated experiments below indicate that even during growth periods a large *reserve store* of hemoglobin producing materials will accumulate in the body due to favorable diet intake. This reserve store of hemoglobin building material in the dog is always to be considered in interpretation of results. When anemia periods are short obviously it may introduce large factors of error and to be sure of one's figures long control periods should follow the initial bleedings to establish the anemia level and exhaust the reserve store.

METHODS. The general plan of procedure was very simple. As opportunity offered litters of pups born in the animal quarters were vaccinated against distemper. Immediately after weaning at 8 weeks of age the pups were divided into groups and fed the diets indicated. A whole milk powder "Klim" was added to all diets—30 grams per day. The growth curves were all normal and pups were active and healthy. Particular attention was given to the feeding of these animals and often individual dogs were fed in separate pens to insure adequate food intake for each dog; however

the weight curve is a fairly safe index. When the diet consisted of salmon bread and some meat product, the liver, kidney or meat was put through a meat grinder and then mixed with water and the salmon bread to a hash. This prevents the dog from picking out any particular material which it may prefer. The pups were fed twice daily up to the age of 4 months and once daily thereafter. The special diet was continued for 10 months or up to the age of 1 year when the dogs were ready for the standard bleeding to ascertain the amount of reserve storage of hemoglobin factors. They have attained their mature weight by this time.

Various details relating to ingredients and preparation of the standard salmon bread, care of animals, technique of bleeding and methods have been carefully described (5). The anemia is produced by cautious bleeding and carried to a hemoglobin level about one-third of normal. If this is done carefully the dogs remain perfectly healthy and active and eat their diets completely. Exhaustion of the reserve store of hemoglobin producing factors usually requires 50 to 60 days and this period was used in all animals included in the tables.

Experimental observations. The standard salmon bread contains wheat flour, potato starch, cane sugar, salmon, canned tomato, cod liver oil, yeast and a salt mixture minus iron, and is an adequate diet for growth or maintenance. This standard salmon bread is not favorable for hemoglobin production in anemia yet it is adequate for the maintenance of hemoglobin production during the growth of pups from weaning (8 weeks) to maturity (1 year of age). There is little storage of hemoglobin building material in the body during salmon bread periods and when we examine table 1 we note the average value for total hemoglobin reserve as 30 grams hemoglobin. The original level of total hemoglobin circulating in the blood is 249 grams or our reserve store is only 12 per cent. Considering the method errors, the activity of the spleen and other unknown variables, we do not lay any stress on this figure. We may say that on a diet of salmon bread the young dog maintains a normal hemoglobin level and perfect health but does not store away in its body much material from which hemoglobin can be fabricated in an emergency.

Method limitations relating to blood volume have been reviewed (1) and we believe the error is greater when the hemoglobin is high and the mixing of red cells and plasma in the smaller vessels is less complete. The plasma volume is reasonably accurate as determined by the brilliant vital red dye method but the red cell volume is calculated on the basis of the hematocrit sample taken from the jugular prior to injection of the dye. Obviously there are possibilities of error here as indicated (1). The total blood volume may be figured as the sum of plasma and red cells and the total circulating hemoglobin is the product of the total volume times the hemoglobin per 1 cc. of blood as given in columns 5 and 9, table 1. As the

dog is made anemic the loss of red cells is made up in large part by an increase in plasma volume but the total blood volume in anemia is decreased 10 to 25 per cent. On the average in the various tables we see that the red

TABLE 1

Salmon bread

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RESERVE
		Blood volume		Hb. per 100 cc. blood	Total circulating Hb	Blood volume		Hb. per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	*cc.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
27–231	12.1	497	629	21.8	248	700	157	5.6	49	168	−31
27–236	16.2	550	602	19.2	223	835	209	4.7	50	167	−6
27–238	14.8	610	560	19.2	227	850	217	6.4	68	216	57
30–114	14.8	720	714	17.8	257	894	239	6.1	69	196	8
30–116	14.6	730	633	17.1	234	812	263	6.1	66	229	61
30–120	16.2	536	683	22.0	269	800	273	5.8	63	210	4
32–5	14.5	690	738	19.3	277	780	254	6.5	69	198	−10
32–6	14.4	640	718	19.2	263	806	326	8.4	97	286	120
33–13	16.2	629	660	19.1	247	941	266	6.2	75	241	69
Av...	14.9	622	660	19.3	249	824	245	6.3	67	212	30

TABLE 2

Mixed kennel diet

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RESERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	*cc.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
24–59	16.9	654	926	17.4	277	946	314	6.5	83	227	33
25–23	15.8	578	648	16.7	206	760	236	6.8	68	165	27
26–18	14.6	776	535	15.5	205	939	255	6.1	73	261	129
26–102	12.9	612	532	17.8	205	807	201	5.8	59	234	88
26–164	12.8	562	574	18.8	216	758	215	6.6	65	187	36
29–65	12.0	520	750	23.0	295	740	184	6.5	60	209	−26
29–66	13.4	391	540	22.4	209	836	221	6.2	66	230	87
29–67	15.7	745	841	20.1	323	929	220	5.7	65	232	−26
29–68	14.4	640	848	21.2	319	926	200	5.4	61	247	−11
Av...	14.4	609	688	19.2	251	849	227	6.2	67	221	37

cell volume decreases about 400 cc. while the plasma volume increases about 200 cc. or the *total blood volume* in the anemic state is about 200 cc. less than in the non-anemic or normal state.

The *total hemoglobin reserve* is readily calculated from the figures given

in the tables. The total anemic circulating hemoglobin (67 gm.) plus the total hemoglobin removed by bleeding (212 gm.) (table 1) averages 279 grams which exceeds the original level of circulating hemoglobin in the non-anemic state (249 gm.) by 30 grams. The *reserve figure* is 30 grams hemoglobin. We may choose to say that some of this 30 grams hemoglobin was held in the spleen and set free as the anemia progressed. It has been shown that there is no reserve of mature red cells in the bone marrow under such conditions (2).

TABLE 3

Kidney and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RESERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	*cc.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
27–239	11.4	541	754	21.4	280	724	234	5.2	50	281	51
27–240	12.3	500	898	23.3	329	845	225	6.1	66	238	−25
27–241	12.3	482	654	21.7	249	714	238	7.2	69	235	55
Av...	12.0	508	768	22.1	286	761	232	6.2	62	251	27

TABLE 4

Skeletal muscle and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RESERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	*cc.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
30–117	15.3	688	762	19.3	283	986	290	6.5	83	330	130
30–118	13.4	531	546	18.5	200	797	251	7.0	74	247	121
30–121	14.7	744	766	18.5	281	886	274	6.8	79	305	103
32–1	13.2	600	642	20.1	252	725	234	6.9	66	217	31
32–3	12.8	610	580	18.6	223	762	224	6.6	66	228	71
Av...	13.9	634	659	19.0	248	831	255	6.8	74	265	91

It is obvious from the weights that these dogs are of a uniform type. They were bred from our anemia stock. These animals are predominantly white bull dog but strains of coach and terrier are represented. Many of the dogs in table 1 were litter mates as indicated by the numbers. Even in the same litter we find considerable differences in the capacity of individual dogs to make hemoglobin on standard diets. This has been touched upon in a study of the marrow (2) in many of the anemia colony dogs.

Table 2 shows results very much like table 1 and the total hemoglobin

reserve averages only 37 grams The kennel diet therefore is adequate for growth and maintenance of the normal hemoglobin level but does not supply a large surplus for reserve purposes. The mixed kennel diet is made up of mixed hospital table scraps and contains much bread, potato and other vegetable material with variable amounts of meat, bones and butter.

Table 3 shows that kidney added to the salmon bread in equal amounts has little effect upon the reserve store of hemoglobin building factors. The average figure for the total reserve store is 27 grams hemoglobin and this is not beyond possible method error. The series is small and no conclusions should be drawn. All three dogs were litter mates and on the average are somewhat smaller than other series. There are individual differences to be noted, especially the high hemoglobin values in the non-anemic period (dog 27–240).

TABLE 5

Liver and salmon bread, equal parts

DOG NO.	WT. AT BEGIN.	NON-ANEMIC				ANEMIC				Hb TOTAL REMOV.	TOTAL Hb RESERVE
		Blood volume		Hb per 100 cc. blood	Total circulating Hb	Blood volume		Hb per 100 cc. blood	Total circulating Hb		
		Plas.	R.B.C.			Plas.	R.B.C.				
	kgm.	*cc.*	*cc.*	*gm.*	*gm.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*
25–97	15.3	735	864	16.8	272	927	356	6.8	88	308	124
27–233	11.5	510	500	19.0	195	760	188	6.5	63	233	101
27–234	14.0	724	625	17.4	237	836	229	6.6	71	307	141
27–235	14.4	512	800	24.6	326	836	206	6.1	64	297	35
30–115	13.0	624	558	16.3	194	809	241	6.1	65	349	220
30–119	13.3	484	381	15.5	135	696	187	5.3	47	233	145
32–2	15.1	765	736	18.2	276	988	182	5.0	59	276	59
32–4	14.1	530	682	20.7	251	800	267	6.9	74	273	96
Av...	13.8	611	643	18.6	236	831	232	6.2	66	285	115

Table 4 shows that skeletal muscle (beef) added to the salmon bread in equal amounts has a significant effect upon the reserve store of hemoglobin building material. The average figure for the total hemoglobin reserve store is 91 grams which is far beyond method or physiological variables. Beef muscle is not as efficient for rapid hemoglobin regeneration as kidney but for the storage of this hemoglobin reserve it seems to be distinctly superior.

Table 5 as might be expected shows that *liver* is the optimum diet factor to bring about a large reserve store of hemoglobin building material in the young animal. The total store of hemoglobin building material represents 115 grams or about one half the original hemoglobin in circulation in the non-anemic state. This represents more than 380 cc. of packed red cells or 760 cc. of normal dog blood and we cannot imagine that the spleen

or bone marrow can account for more than a small fraction of this large reserve store. One may speculate as to the form and location of this store. We suspect that a large part is stored in the liver, but work in progress may throw light on this point. The protein reserve which is related to the plasma protein output (3) may also be concerned or there may be a common protein reserve store which is mobilized in emergencies either toward hemoglobin or plasma protein.

SUMMARY

In normal growing dogs from weaning (8 weeks of age) to the adult state (1 year of age) there is ample hemoglobin building material for maintenance but there is little reserve storage of these hemoglobin producing substances (30 gm. hemoglobin) when the diet consists of standard salmon bread.

When skeletal muscle (beef) is added to the basal ration there is demonstrable a considerable reserve store of hemoglobin producing material—91 grams hemoglobin.

With liver feeding during this growth period the dog will store away a large supply of material out of which new hemoglobin can be produced in an emergency—115 grams hemoglobin.

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THE CHEMICAL MEDIATION OF SYMPATHETIC VASODILATOR NERVE IMPULSES

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Received for publication January 30, 1935

Dale and Feldberg (1934) have recently emphasized the probability that, although sympathetic nerve impulses are usually mediated by the adrenine-like sympathin, some may act through liberation of acetylcholine. Dale (1934) suggests that the former be called adrenergic and the latter cholinergic.

That in appropriate experimental conditions adrenine may evoke vasodilatation is well known. The vasodilator effects of acetylcholine likewise are well known. It appeared to us important to determine whether the widely distributed sympathetic dilators in the cat and dog are adrenergic, or cholinergic, or both, and whether adrenine exerts its depressor effects directly or through liberation of acetylcholine.

The only tests available for distinguishing between adrenine and acetylcholine in the amounts which are liberated by stimulation of autonomic nerves are pharmacological. We therefore studied the effects of ergotoxine and atropine, cocaine or eserine, on the blood-pressure changes caused by acetylcholine, adrenine and the stimulation of sympathetic nerves in the cat and dog. The rabbit also was examined because of its peculiar reactions to adrenine—no depressor effects from small doses of the hormone and no "reversal" of the pressor into depressor effects by ergotoxine (Dale, 1906).

METHOD. The animals were anesthetized with either dial or urethane or they were rendered spinal by destruction of the brain while under ether anesthesia. The blood pressure was recorded from a carotid or a femoral artery by means of a mercury or a Hürthle manometer. The drugs were injected intravenously. The nerves (usually the lower abdominal chains, crushed or severed centrally) were stimulated with a Harvard inductorium through buried shielded electrodes.

RESULTS. A. *Ergotoxine in the cat.* The depressor effect of adrenine after ergotoxine is a well-established fact. Stimulation of sympathetic nerves, e.g., the lower abdominal chains, which previously induced a rise of blood pressure, will then evoke a fall which is usually succeeded by a rise. The initial fall has been interpreted as due to the effects of sympathetic

dilators (Dale, 1906; Cannon and Rosenblueth, 1933). In numerous experiments we have invariably found that after ergotoxine acetylcholine is quite effective in evoking depressor responses.

Because ergotoxine consistently conditions a depressor response to stimulation of sympathetic fibers distributing to peripheral structures (skin and muscles), it was routinely injected and the other pharmacological tests, to be described in the succeeding sections, were thereafter applied.

B. *Cocaine in the cat.* The depressor effects of adrenine (commercial adrenalin) after ergotoxine are augmented by cocaine in doses of 8 mgm. per kgm. (fig. 1). The responses to acetylcholine are not much nor consistently altered by cocaine (fig. 2). As regards the action of cocaine on the complex of responses to stimulation of the lower abdominal sympathetic chains after ergotoxine, the initial fall is either not changed or may be decreased, while the subsequent rise is usually augmented (fig. 3).

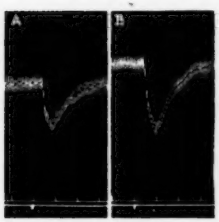

Fig. 1

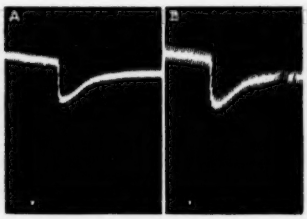

Fig. 2

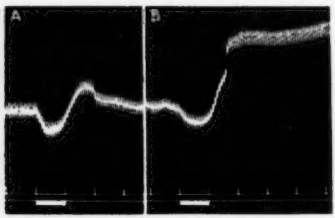

Fig. 3

Fig. 1. Augmenting effect of cocaine on the fall of blood pressure evoked by adrenine after ergotoxine. Spinal cat under curare. Ergotoxine (4 mgm. per kgm.). At the signals 0.2 cc. adrenine (1:100,000) injected intravenously. Time intervals in this and the succeeding figures: 30 seconds.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

Fig. 2. Negligible effects of cocaine on the fall of blood pressure elicited by acetylcholine. Cat under dial anesthesia. Ergotoxine (4 mgm. per kgm.). At the signals 0.1 cc. acetylcholine bromide, 1:1,000,000.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

Fig. 3. Effects of cocaine on the responses to stimulation of the lower abdominal sympathetic chains after ergotoxine (2 mgm. per kgm.). Cat under dial anesthesia. At the signals the nerves were stimulated with the same intensity.

A. Before cocaine.

B. After the injection of cocaine (8 mgm. per kgm.).

C. *Atropine in the cat.* Atropine (1 mgm. per kgm.) does not prevent the depressor effect of adrenine after ergotoxine (fig. 4). On the other hand, the influence of acetylcholine on the blood pressure, even when given in doses as large as 0.001 mgm., is wholly abolished by atropine.

The effect of atropine on the results of sympathetic nerve stimulation are complex and depend largely on the anesthetic employed. If the cat is under dial anesthesia the dose of atropine mentioned above merely decreases the initial fall, leaving the delayed rise unimpaired (fig. 5). In decapitate animals, to which curare and ergotoxine have been administered, atropine may eliminate entirely the initial drop of pressure, and the delayed rise may be replaced by a slight prolonged fall (fig. 6) with subsequent recovery. This delayed fall was especially marked when the hypogastric nerves were stimulated.

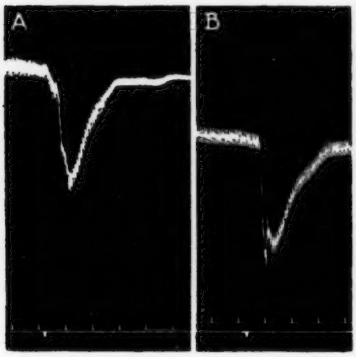

Fig. 4

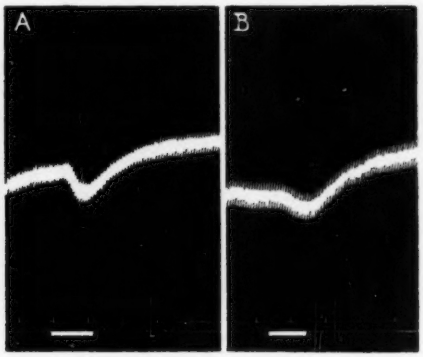

Fig. 5

Fig. 4. Negative effects of atropine on the fall of blood pressure elicited by adrenine after ergotoxine. Spinal cat under curare. Ergotoxine (4 mgm. per kgm.). At the signals 0.1 cc. adrenine, 1:100,000.

A. Before atropine.

B. After the injection of atropine (1 mgm. per kgm.).

Fig. 5. Persistence after atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a cat under dial anesthesia and injected with ergotoxine and cocaine. At the signals the nerves were stimulated with the same intensity.

A. Before atropine.

B. After the injection of atropine (1.2 mgm. per kgm.).

D. *Eserine in the cat.* This drug (0.1 to 0.2 mgm. per kgm.) has no influence on the vascular responses to adrenine. The marked augmenting action of eserine on the effects of acetylcholine is too well known to require any illustration. The initial fall of arterial pressure on stimulation of the lower abdominal sympathetic chains after ergotoxine may be uninfluenced or definitely enhanced by eserine; the delayed rise is usually unaffected (see fig. 7 for the similar phenomenon in the dog).

E. *Curare.* Since this drug was administered to the spinal cats in order

to avoid the convulsant effects of ergotoxine and eserine, it was important to determine its influence on the responses studied. This influence was found to be practically negligible as affecting the results of injecting adrenine or stimulating the nerves, while there was usually a definite decrease in the drop of blood pressure elicited by acetylcholine.

F. *The dog.* The action of ergotoxine and cocaine in dogs was similar to that noted in cats. The effects of eserine in enhancing the responses

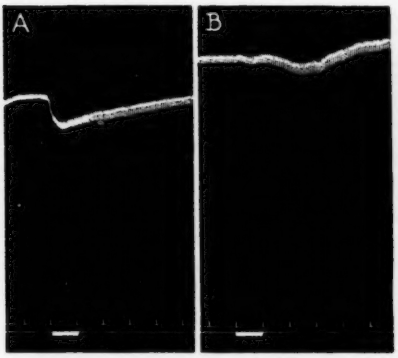

Fig. 6

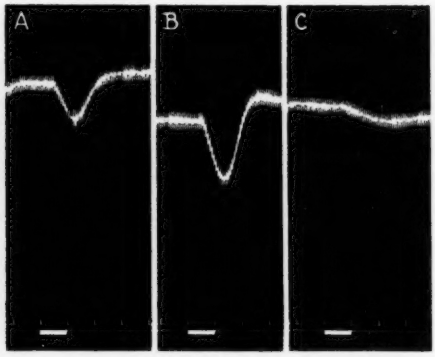

Fig. 7

Fig. 6. Abolition by atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a spinal cat injected with curare, ergotoxine and cocaine. At the signals the nerves were stimulated with the same intensity.

A. Before atropine.

B. After injection of atropine (1 mgm. per kgm.).

Fig. 7. Increase by eserine and abolition by atropine of the initial fall of blood pressure evoked by stimulation of the lower abdominal sympathetic chains in a dog under dial anesthesia and injected with ergotoxine. At the signals the nerves were stimulated with the intensities noted below.

A. Before eserine and atropine. Coil distance 8 cm.

B. After injection of eserine (0.1 mgm. per kgm.). Coil distance 8 cm.

C. After injection of atropine (1 mgm. per kgm.). Coil distance 7 cm.

to acetylcholine are especially striking in the dog, since before eserine relatively large doses of acetylcholine are necessary to obtain any depressor response. For example, in one instance, after ergotoxine, 0.5 cc. acetylcholine (1:100,000) caused a fall of blood pressure from 144 to 136 mm. Hg; after eserine (0.1 mgm. per kgm.) 0.05 cc. acetylcholine in the same dilution reduced the pressure from 130 mm. Hg to 70. Eserine increases also the initial fall attending nerve stimulation (fig. 7A and B).

Atropine in a dose of 1 mgm. per kgm., which does not abolish the initial

fall of blood pressure on stimulation of the lower abdominal sympathetic chains in cats under dial and given ergotoxine (section C, fig. 5), does readily abolish this initial depressor effect in dogs under dial (fig. 7C).

G. *The rabbit.* Ergotoxine induces a fall of blood pressure in rabbits under urethane, instead of the rise invariably obtained in cats under various anesthetics. As mentioned in the introduction, Dale (1906) showed that after ergotoxine adrenine does not induce a fall of blood pressure in rabbits as it does in cats. We readily confirmed this fact. Stimulation of the lower abdominal sympathetic chains in the same experimental conditions failed invariably to elicit any fall of blood pressure, whether initial or delayed. Administration of cocaine did not modify the negative results.

H. *Influence of the anesthetic.* The difference of effects of atropine when injected into spinal cats or into cats anesthetized with dial has been mentioned (section C). A further difference between these two preparations is that ergotoxine is more efficient in paralyzing pressor responses to adrenine or to nerve stimulation in spinal, curarized cats than in cats under dial (cf. Cannon and Rosenblueth, 1933). In the present experiments this difference was again noted, especially in the delayed effects of stimulation of the lower abdominal sympathetic chains. In animals under dial this delayed effect was usually a rise and atropine had little influence on it. In the spinal animals, on the other hand, there was frequently no delayed rise after ergotoxine, the preliminary fall being prolonged into a slow gradual recovery, and the delayed fall after atropine was particularly prominent and easy to obtain.

Discussion. The object of these experiments was to obtain information regarding the adrenergic or cholinergic nature of sympathetic dilators. Pharmacological tests had to be employed, as previously remarked, because they alone were available. We feel, however, that only tentative conclusions may be derived from the data presented, because these data seem to us to illustrate how untrustworthy pharmacological evidence may be and how cautiously we should proceed before drawing any physiological inferences therefrom.

If cats and dogs had been observed solely *under dial anesthesia* a striking difference would have appeared as regards the action of atropine (sections C and F). Since the same doses of this drug (1 mgm. per kgm.) abolish the initial fall of blood pressure resulting from sympathetic stimulation after ergotoxine in the dog, and not in the cat, one might conclude that there is a cholinergic dilator component in the abdominal sympathetic of the dog which is absent or minimal in the cat. When, however, the same dose of atropine is given to spinal cats, the initial fall disappears entirely (section C), showing that the difference may be only quantitative and due to specific variation in susceptibility to atropine in the two experimental conditions, and not to differences in the chemical mediation of the nerves involved.

Further, when the inference is drawn that a response is mediated by acetylcholine because it is impaired or abolished by atropine, the tacit assumption is made that atropine is without influence on the effects of adrenine or sympathin. Such an assumption may be incorrect (cf. Magnus, 1908 and Gasser, 1930).

From the data presented, however, certain conclusions appear to be justified. The depressor action of adrenine is probably a direct effect, and not due to liberation of acetylcholine. In support of this view is the increase of the responses by cocaine (fig. 1), the persistence after atropine (fig. 4) which abolishes the action of acetylcholine, and the lack of augmenting action of eserine (see section D). The effect, furthermore, is probably specific (i.e., adrenine acts on specific vessels which possess an adequate receptive mechanism) and not a generalized action on any vessels. In support of this statement is the absence of depressor effects in rabbits under conditions which in cats and dogs invariably yield a fall of blood pressure.

These views concerning the depressor action of adrenine, together with an application of Elliott's law, lead us to expect that certain of the vasodilator sympathetic fibers in the cat and dog should be adrenergic. We should also expect no adrenergic dilators in the rabbit. That the blood pressure does not fall on stimulation of the lower abdominal sympathetic chains in the rabbit, given ergotoxine (section G), confirms this expectation. It further leads to the conclusion that cholinergic sympathetic dilators are likewise absent—i.e., that there are *no* sympathetic dilators to the hind limbs or tail in the rabbit.

The initial fall on sympathetic stimulation in the dog and the spinal cat, after ergotoxine, is abolished by atropine (figs. 6 and 7C), is not consistently modified by cocaine (fig. 2), and is increased by eserine (fig. 7B). It seems probable, therefore, that there is a cholinergic component partly responsible for the effect. Bülbring and Burn (1934), working with perfused limbs of cats and dogs, report that in the dog eserine increases the dilator effects of sympathetic stimulation and atropine abolishes them, whereas in the cat the responses are unaffected by either drug. They conclude that cholinergic sympathetic dilators are present in the dog and absent in the cat. This discrepancy between Bülbring and Burn's results and inferences and our own may be due to the variability of the preparation which they employed to demonstrate sympathetic dilators. Indeed, they report irregular results from sympathetic stimulation in the perfused limbs, constriction instead of dilatation being often the response. They further had to use adrenine in their perfusing fluid in order to permit a dilatation to occur, and, as pointed out above (see p. 34), atropine may not be without action on the responses which involve adrenine. Finally, it would appear from our data that atropine is relatively more effective in dogs than in cats in block-

ing sympathetic dilators (sections C and F). The differences observed by Bülbring and Burn may then again be merely quantitative, not qualitative.

If cholinergic sympathetic dilators are present in the cat and dog the question again arises whether there are any adrenergic ones, as Elliott's law would lead us to expect. We believe that the instances of a *delayed* fall of blood pressure (see figs. 6B and 7C, and section C) favor an affirmative answer to this question. It must not be forgotten that, as we have previously noted (Cannon and Rosenblueth, 1933), the paralyzing effects of ergotoxine on constrictor sympathetic impulses are relative, dependent on the dose of ergotoxine and on the anesthetic employed (cf. section H). When we stimulate, after ergotoxine, a mixed sympathetic trunk, containing both constrictors and dilators, the constrictors may still have some immediate local effect—smaller, however, than before the ergotoxine. The changes of blood pressure will then depend on the relative importance of the constriction and dilatation. When the cholinergic dilators are present, before atropine, an immediate fall occurs. Indeed, the briefness of this fall (figs. 3, 5 and 7) is in harmony with the rapid destruction of acetylcholine and the correspondingly rapid disappearance of cholinergic responses. When the cholinergic dilators have been blocked by atropine, the blood-pressure changes will depend on the balance of adrenergic constriction and dilatation, local and general (sympathins E and I). The fact that falls are more frequent and significant with larger doses of ergotoxine and in spinal cats than in cats under dial (section H) is in favor of the presence of adrenergic dilators.

Since, however, atropine not only may abolish the initial, presumably cholinergic, fall of blood pressure but also frequently converts a delayed rise into a delayed fall, we are finally led to conclude that atropine either augments the effects of sympathin I, or impairs the responses to sympathin E, or both.

SUMMARY

In anesthetized cats, dogs and rabbits, injected with ergotoxine, the changes of blood pressure evoked by adrenine, acetylcholine and stimulation of the lower abdominal sympathetic chains were recorded before and after cocaine, eserine and atropine.

In cats and dogs cocaine increases the fall caused by adrenine (fig. 1) and the delayed rise elicited by sympathetic stimulation (fig. 3); eserine increases the initial fall elicited by sympathetic stimulation (fig. 7B); and atropine diminishes (fig. 5) or abolishes (figs. 6 and 7C) this initial fall and may change the delayed rise into a slight delayed fall (figs. 6 and 7C). In rabbits stimulation of the lower abdominal sympathetic chains does not evoke a fall of blood pressure after ergotoxine.

It is concluded that the effects of adrenine are specific and not mediated by acetylcholine (p. 38); that the sympathetic contains some cholinergic (p. 38) and some adrenergic (p. 38) vasodilators in cats and dogs, and no vasodilators at all in the rabbit (p. 38).

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EVIDENCE FOR A POTASSIUM SHIFT FROM PLASMA TO MUSCLES IN RESPONSE TO AN INCREASED CARBON DIOXIDE TENSION

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Received for publication February 5, 1934

Complete osmotic equilibrium can be maintained across a water permeable membrane by one of three methods: 1, complete impermeability to electrolytes; 2, complete impermeability to anions or to cations; 3, maintenance of hydrostatic pressure to balance the osmotic pressure. There are three membranes in the body which are of special importance from this point of view: the membrane of the red blood corpuscle, that of the tissue cells, and that of the capillary walls. Of these three, the last is permeable to all electrolytes and must maintain its complete equilibrium by the maintenance of a capillary blood pressure to balance the colloid osmotic pressure of the blood. Osmotic equilibrium across the red blood corpuscle membrane is maintained by its impermeability to cations. There is evidence in muscle cells that the equilibrium is maintained by complete impermeability to anions.

Across the red blood corpuscle membrane there is a shift of chloride from plasma to corpuscles when the carbon dioxide tension is increased. If the muscle cell is permeable to cations rather than to anions, a similar increase in carbon dioxide tension should result, not in a chloride shift, but in a potassium shift, since apparently the potassium is the only cation normally present which can penetrate (except the H^+ ion). The direction of this shift will naturally depend on the relative buffering capacities of the cells and plasma. If an increase in carbon dioxide tension causes a greater increase in acidity in the cells than in the plasma, then potassium will tend to shift from the plasma to the muscles in order to neutralize this acidity. If the buffering power of the plasma is artificially diminished by substituting Ringer's solution, then the same increase in carbon dioxide tension should increase the acidity of the solution more than the acidity of the cells and cause potassium to go from the cells to the solution.[1] Since the muscles

[1] In our former paper (1934) we erroneously predicted a movement of potassium from cells to plasma as a result of increased carbon dioxide tension because of failure to realize that blood is better buffered than muscle.

are impermeable to anions no chloride shift would be expected between the blood and the cells.

As far as the red blood corpuscles are concerned, this theory may be regarded as well established. For the muscle cell it may probably be regarded as merely an hypothesis, although a good deal of evidence has been accumulated in its favor. The following experiments were designed to add to this evidence and they serve to show that in the sartorius muscle immersed in its own blood an increase of carbon dioxide tension causes potassium to move from the plasma to the muscles as expected, while the reverse is found in Ringer's solution. They show further that there is no chloride shift to be detected.

METHOD. The experiments were performed on frogs (*Rana pipiens*). The brain was crushed with a strong hemostat, the heart exposed and the blood drawn directly from the aorta into a syringe containing about 1 mgm. of heparin. Between 1 and 2 cc. of blood could be obtained from each frog. Sartorius muscles were dissected out and immersed in this blood equilibrated at different carbon dioxide tensions. In some cases, the blood from several frogs was pooled and the muscles were equilibrated in a 25 cc. flask which was rotated slowly in a horizontal position in the water bath at 22°C. In other experiments the blood from one frog was divided equally between two test tubes (by counting drops from the syringe) and two sartorius muscles were immersed, one in each test tube. The tubes were agitated gently in the water bath to prevent settling of the blood corpuscles. After a period, usually 5 hours in duration, the muscles were removed, weighed again on a torsion balance and set aside for analysis. The blood was centrifuged without exposure to air and the plasma was analyzed either for chloride or for bicarbonate.

Potassium was analyzed by the method of Shohl and Bennett (1928) as previously described (Fenn and Cobb, 1934) with one modification introduced by Dr. J. I. Thaler in this laboratory (unpublished). This consisted in separating out the precipitate of potassium chloroplatinate by centrifuge instead of by filter since great difficulty was experienced in finding reliable filters. This procedure did not seem to give the occasional large errors which caused Shohl and Bennett to discard the use of the centrifuge. On the contrary, in our experience, the new method is to be preferred because it eliminates occasional large errors met with in using filters and obviates the necessity of testing the filter each day on a known solution. The methods used for chloride analyses have been previously described (Fenn, Cobb and Marsh, 1934).

RESULTS. *Control analyses.* A series of control analyses of matched muscles are collected in table 1. The tibialis anticus longus and the semitendinosus were included because they have about the same weight as the sartorius muscle. The muscles were moistened with Ringer's solution

during dissection, then dipped momentarily in that solution, blotted, weighed on a torsion balance and analyzed. The average difference between the two analyses made on a pair of muscles was 2 per cent, and the probable error in analyzing matched muscles is 0.115 m.-eq. per cent or 1.4 per cent. These analyses with the new method are somewhat better than those previously reported (Fenn and Cobb, 1934).

Potassium changes during immersion in frog blood. When frog sartorius muscles are dissected out and immersed for 5 hours in Ringer phosphate solution at 22°C. and pH 7.2 they lose about 10 per cent or more of their potassium and this loss increases with increase in acidity and vice versa (Fenn and Cobb, 1934). Believing that this loss of potassium was due to

TABLE 1

Control series

Potassium contents of matched muscles

(Milli-equivalents per 100 grams of muscle)

MUSCLE	RIGHT	LEFT	DIFFERENCE
Sartorius	7.88	8.08	+0.20
	8.24	8.28	+0.04
	7.78	7.98	+0.20
	8.72	8.62	−0.10
Semitendinosus	7.88	8.16	+0.28
	7.93	7.85	−0.08
	7.80	7.70	−0.10
	8.03	7.85	−0.18
Tibialis anticus longus	7.44	7.19	−0.25
	7.68	7.80	+0.12
	7.88	7.72	−0.16
Average	7.933	7.930	−0.003

the abnormality of the Ringer's solution compared to frog blood we anticipated that if the muscles were immersed in the blood of the same animal equilibrated with carbon dioxide at normal tensions (about 5 per cent) there would be complete potassium equilibrium. We also anticipated that if the carbon dioxide tension were further increased the increase in acidity in the muscle would be greater than the increased acidity in the blood (due to the lower buffering capacity of the muscle) and potassium would move from the blood into the muscle—and vice versa at lower carbon dioxide tensions.

To test this theory the experiments of table 2 were carried out. The experiments consisted in dissecting out the two sartorius muscles of one frog, analyzing one of the muscles immediately as a control and analyzing

the other after it had been immersed for 5 hours in blood equilibrated with different carbon dioxide tensions. The results were contrary to expectations for they showed that at all carbon dioxide tensions from 0.7 to 18.6 per cent there was a loss of potassium from the muscle to the blood amounting on the average to 2.5 per cent of the amount present. The absolute amount lost, 0.26 m.-eq. per cent is 9.3 times the probable error of the mean

TABLE 2

The potassium content of matched muscles before and after immersion for 5 hours at 22°C. in frog blood plus CO_2 and O_2

(M-eq. per 100 grams initial weight)

EXPERIMENT	CO_2	Δ WEIGHT	POTASSIUM CONTENT		Δ K
			Before	After	
	per cent	*per cent*			
1 a	0.7	−0.8	9.00	8.67	−0.33
b	0.7	−4.0	8.52	7.93	−0.59
c	0.7	+1.7	8.80	8.77	−0.03
2 a	1.65	−7.0	8.50	8.30	−0.20
b	1.65	−5.1	9.08	8.98	−0.10
3 a	4.93	−1.0	9.18	8.21	−0.97
b	4.93	−3.0	9.00	8.36	−0.64
c	4.93	−2.1	9.06	8.03	−1.03
4 a	5.0	−2.0	8.87	9.56	+0.69
b	5.0	−7.0	8.82	9.00	+0.18
c	5.0	−9.0	8.75	8.93	+0.18
5 a	9.28	−3.3	8.49	8.82	+0.33
b	9.28	+0.8	9.52	9.02	−0.50
c	9.28	+1.4	9.06	8.85	−0.21
6 a	18.65	0	8.80	7.98	−0.82
b	18.65	0	8.82	8.10	−0.72
c	18.65	−4.0	7.65	8.00	+0.35
Average.....		−2.6	8.82	8.56	−0.26

All muscles weighed between 92 and 140 mgm.

of 17 determinations. In five of the 17 muscles there was a gain in potassium, the amount of the gain being in three cases at least significantly greater than the analytical error. In spite of this evidence of variability due to uncontrolled factors, it must be concluded that under the conditions of these experiments any tendency of the muscles to gain potassium because of high carbon dioxide tension is more than balanced by a tendency to lose potassium due to other factors such as injury to the fibres. Whatever

this loss may be due to it is only about one-quarter as great when the muscles are immersed in blood as when they are immersed in Ringer's solution.

In a further attempt to elucidate the rôle of carbon dioxide in the potassium equilibrium, we carried out the experiments listed in table 3, the object being to soak both muscles in blood, usually at 1 per cent carbon dioxide tension, for a short preliminary period in order to "wash away" any

TABLE 3

Potassium contents of paired muscles and nerves

EXPERIMENT	PRELIMINARY PERIOD		EXPERIMENTAL PERIOD		Δ WEIGHT		POTASSIUM CONTENT		Δ K
	Time	CO_2	Time	CO_2	Control	Experimental	Control	Experimental	
				Muscle					
	hrs.	*per cent*	*hrs.*	*per cent*	*per cent*		*m.-eq. per 100 gm. initial wt.*		
1	0.5	9.3	5	9.3	−3.6	+2.4	7.57	8.11	+0.54
2	0.5	9.3	5	9.3	−2.9	0	8.24	8.44	+0.20
3	0.5	9.3	5	9.3	−1.0	−1.0	8.57	8.59	+0.02
4	2.7	1.0	3.3	9.3	+4.0	+8.6	8.14	8.22	+0.08
5	2.6	1.0	3.3	9.3	+0.8	+3.4	6.98	7.88	+0.90
6	2.5	1.0	3.3	9.3	+4.3	+1.9	7.34	7.11	−0.23
7	2.6	1.0	4.2	17.2	−3.5	−7.1	8.00	8.34	+0.34
8	3.3	1.0	3.1	26	0	+7.1	8.52	8.96	+0.44
9	3.2	1.0	3.3	26	−4.2	−0.8	8.24	7.37	−0.87
10	3.0	1.0	3.1	26	−2.2	−5.2	8.72	8.28	−0.44
Average							8.03	8.13	+0.10
				Nerve					
11	1.8	1.0	3.2	9.3	−1.0	+8.5	3.55	3.43	−0.12
12	1.5	1.0	4.2	9.3	−10.4	−13.5	2.92	3.50	+0.58
13	2.7	1.0	3.3	9.3	−7.6	−4.0	3.02	3.63	+0.61

Both control and experimental muscles and nerves were immersed in blood-CO_2 for a preliminary period after which the control muscle was weighed and analyzed. The experimental muscle or nerve was then immersed for an experimental period in blood-CO_2, then weighed and analyzed. Muscles weighed 85 to 135 mgm. and nerves 89 to 101 mgm.

potassium due to injury, and to study in a subsequent experimental period the movements of potassium under the influence of different carbon dioxide tensions. At the close of this preliminary period the control muscle was analyzed for potassium and the carbon dioxide tension was increased for the experimental period after which the experimental muscle was also analyzed. Three experiments of this sort were also carried out on nerves, the sciatic nerves from three frogs being used for each experiment.

The results of these experiments, listed in table 3, are somewhat more encouraging. As shown in the last column of table 3, all but three of the ten muscles and two out of three of the nerves showed a gain of potassium during the experimental period. The average gain for the muscles was 0.1 m.-eq. per cent which is approximately three times the probable error of the mean of ten such observations.

In studying the time course of potassium intake by muscles in alkaline Ringer's solution of high potassium content we have found (1934) that an initial gain is followed by a terminal loss of potassium. In interpreting the results of table 3 it may be supposed that the three muscles which showed a loss of potassium had reached the beginning of this terminal stage while most of the others were still near the peak of the intake. It is to be supposed also that at lower carbon dioxide tensions this intake of potassium would not occur. On this basis the results may be taken to indicate that high carbon dioxide tension causes a potassium shift to the cells. The same interpretation may also be applied to the three nerve experiments. The error of the analyses is probably larger here, however, for the absolute amounts of potassium to be measured are less; this probably explains the small loss in one experiment, the gains in the other two cases being much larger.

We do not, however, regard the data of table 3 as a perfectly satisfactory proof of a potassium shift to the cells caused by carbon dioxide. Aside from the smallness of the average gain observed it would be necessary to show also that at low carbon dioxide tensions under otherwise similar conditions there would be no such intake of potassium. Instead of attempting to prove this point we have undertaken a more direct proof by comparing the potassium content of matched muscles immersed in blood for 5 hours at 22°C., one at low and the other at high carbon dixide tensions. Any factors which cause a loss of potassium due to the disturbance of the normal equilibrium *in vivo* are then equal in the two cases and any difference in potassium content after the period of immersion in the blood must be attributed to the effect of carbon dioxide.

Muscle potassium under high and low carbon dioxide tensions. The results of these experiments are shown in table 4. Two similar experiments on nerve (3 pairs for each experiment) as well as one experiment with 3 pairs of muscles in Ringer's solution instead of blood are also included. The differences in potassium content of the muscles or nerves in the two solutions are shown in the last column, a plus sign indicating more potassium in the muscle or nerve in high carbon dioxide. In 17 out of 20 muscles in blood and in both nerve experiments, the high carbon dioxide resulted in a higher potassium content. The average gain in potassium due to carbon dioxide in the muscles in blood was 0.5 m.-eq. per cent. This is a 6 per cent difference between the two muscles and is 20 times the probable error of the mean of 20 determinations. In other words high carbon

TABLE 4

The effect of carbon dioxide on the potassium equilibrium of muscle and nerve after immersion for 5 hours at 22°C.

EXPERIMENT	CO_2 TENSION		Δ OF WEIGHT		K CONTENT		K EXCESS DUE TO CO_2
	a	b	a	b	a	b	b − a
Muscle in blood							
	per cent	*per cent*	*per cent*	*per cent*	*m.-eq. per 100 gm. initial wt.*		
1	1.01	9.28	−6.0	−3.8	6.93	8.16	+1.23
			−0.9	+5.1	7.57	8.32	+0.75
			−5.6	+0.8	6.70	8.49	+1.79
2	4.93	19.32	−1.9	0	8.59	9.03	+0.44
			0	0	8.92	9.23	+0.31
			−11.3	−6.8	7.93	8.57	+0.64
3	0.89	9.28	−8.5	−7.0	7.32	7.52	+0.20
			−2.6	−1.6	8.29	8.42	+0.13
			−4.1	−4.3	8.16	7.85	−0.31
4	1.6	14.3	−2.7	−2.8	8.24	9.46	+1.22
			−5.4	−3.5	8.08	7.62	−0.46
			−7.4	−5.0	7.78	8.80	+1.02
5	0.7	40.0	−4.2	−4.1	7.66	7.62	−0.04
			−6.1	−0.8	7.51	8.05	+0.54
			0 *	+1.6	7.51	7.87	+0.36
			−2.5	+0.8	7.15	7.80	+0.65
			−1.7*	+8.3	7.77	7.85	+0.08
6	5.0	5.0†	−2.3	−3.1	8.01	8.08	+0.07
			−4.1	−5.2	8.26	8.42	+0.16
			−5.2	−5.5	7.70	8.18	+0.48
Average					7.80	8.3	+0.50
Muscle in Ringer's solution							
7	1.0	9.28	+1.9	+1.9	7.75	7.37	−0.38
			−5.6	−5.6	6.98	6.55	−0.43
			−10.0	−13.1	7.19	6.96	−0.23
Average					7.31	6.96	−0.35
Nerve in blood							
8	1.6	14.3	−5.1	0	4.37	4.53	+0.16
9	1.2	9.28		−4.0	4.58	5.09	+0.51

* Semitendinosus muscles.

† NaH_2PO_4 added to reduce combined CO_2 from 63 to 42 volumes per cent.

dioxide tension has either caused potassium to move from the plasma to the muscles or at least hindered the diffusion of potassium from muscles to plasma. In experiment 6 the carbon dioxide tension was not varied but instead acid sodium phosphate was added to one-half of the blood, thus reducing its bicarbonate content, when equilibrated with 5 per cent carbon dioxide, from 63 to 42 volumes per cent. In this case also the addition of acid to the blood caused an increase of potassium in the muscles. In experiment 7 Ringer's solution was substituted for blood and the result was exactly opposite, that is, an increase of carbon dioxide accelerates the diffusion of potassium out of the muscles. Further experiments of this type were not repeated because they merely served to confirm our previous experiments on this point (Fenn and Cobb, 1934). The two experiments with frog sciatic nerves in table 4 show that in nerves also, high carbon dioxide tension causes a diffusion of potassium from plasma to nerves. Proof of similarity between nerve and muscle in this respect was lacking in our former work on nerve electrolytes (Fenn, Cobb, Hegnauer and Marsh, 1934).

In the course of these experiments we have usually centrifuged the blood under oil after removal of the muscles and have determined the carbon dioxide content of the "true" plasma at different carbon dioxide tensions by the Van Slyke manometric method. These data are plotted in figure 1 together with similar carbon dioxide dissociation curves for sartorius muscle (from Fenn, 1928) and for our Ringer-phosphate solution. The intermediate position of muscle between blood and Ringer is very evident. When the carbon dioxide tension increases from 1 per cent to 10 per cent. the change in H^+ ion concentration calculated roughly from this graph (if $pK_1 = 6.17$) is 73×10^{-8} in Ringer, 29×10^{-8} in muscle and 9.6×10^{-8} in true plasma. Hence if K^+ diffuses by exchanging with H^+ it should move toward the solution where the increase in H^+ is greatest, i.e., from plasma to muscle and from muscle to Ringer. This is actually what happens, as shown in table 4.

Potassium content and carbon dioxide capacity. If the change in potassium content of the muscles which was produced by high and low carbon dioxide tensions is a true potassium shift it must represent, not a diffusion of potassium as a salt, but an exchange between K^+ and H^+, or some other cation, just as the chloride shift represents an exchange of anions. If this is the case there must be corresponding changes in the carbon dioxide binding capacity of the muscle. The following measurements of the combined carbon dioxide have shown that this expectation is realized.

In each experiment one sartorius and one ileofibularis muscle were immersed together in 1 cc. or more of frog blood or Ringer equilibrated with 0.5 per cent carbon dioxide, while the matched muscles were in an equal amount of the same solution, equilibrated with 20 per cent carbon dioxide.

After 5 hours at 22°C. the muscles were removed from the blood, dipped momentarily in Ringer's solution, blotted, weighed, then dipped momentarily in unbuffered Ringer's solution to moisten them and put into respirometers containing 12 per cent citric acid in the side arms but no other solutions. Further ionic interchanges were therefore impossible.

Since the normal carbon dioxide tension in frogs is around 5 per cent there is presumably a synthesis of phosphocreatine in 0.5 per cent carbon dioxide and a breakdown in 20 per cent carbon dioxide (Root, 1933). When both sets of muscles are returned to 5 per cent carbon dioxide in the respirometer the reverse processes apparently occur as indicated by the volume changes recorded during the ½ to 2 hours previous to acidification.

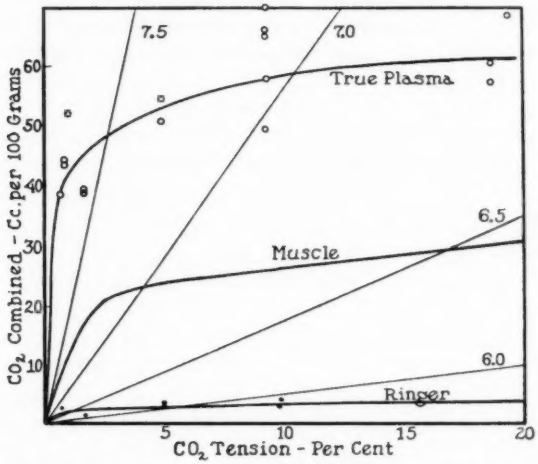

Fig. 1. Carbon dioxide dissociation curves of plasma, muscle and Ringer to show the relative buffering capacities.

The magnitude of these volume changes during equilibration are included in table 5, the minus sign indicating an absorption of carbon dioxide and vice versa. Before tipping the acid we usually waited 2 hours until these changes (presumably of phosphocreatine) were complete. As shown in table 5 the muscles previously in 20 per cent carbon dioxide gave the higher carbon dioxide capacity in blood and the lower carbon dioxide capacity in Ringer. It might be argued that the result in blood was due to the phosphocreatine breakdown in 20 per cent carbon dioxide which had not been completely reversible but this should have then been true in Ringer's solution also. Evidently the carbon dioxide capacity has been varied by ionic interchanges which are irreversible in the respirometer because there is no outside solution. Had we waited longer for the completion of phos-

phocreatine changes before acidification the differences observed in Ringer muscles would have been slightly greater and the differences in blood muscles slightly less but the sense of the results would have been unchanged. Actually there was very little difference between the rates of volume change in the respirometers at the time of acidification.

On the average, previous treatment with 20 per cent carbon dioxide in blood caused an increase of 9 volumes per cent in the carbon dioxide capacity. This equals 0.4 m.-eq. and may be compared with the average excess potassium found under these conditions in table 4 of 0.5 m.-eq. per 100 grams of muscle. Likewise previous treatment with 20 per cent carbon dioxide in Ringer's solution decreased the carbon dioxide capacity on the average 2.9 volumes per cent or 0.13 m.-eq. which may be compared with the average deficiency of potassium of 0.35 m.-eq. under similar conditions from table 4. This comparison is not quantitatively exact but

TABLE 5

The effect of carbon dioxide on the alkali reserve of muscle

(Cc. of CO_2 combined in 100 grams of muscle equilibrated with 5 per cent CO_2)

SOLUTION	AFTER 5 HOURS IN 0.5 PER CENT CO_2	AFTER 5 HOURS IN 20 PER CENT CO_2
Frog blood	18.4	26.4
Frog blood	17.9	29.9
Frog blood	20.2	27.0
Average change during equilibration.....	−6.6	−0.8
Ringer-phosphate	8.1	7.0
Ringer-phosphate	11.1	6.7
Ringer-phosphate	8.2	4.9
Average change during equilibration.....	−3.5	+0.3

the agreement is close enough to suggest that the changes in potassium content at least during immersion in blood are due chiefly to exchanges between K^+ and H^+ or to diffusion of potassium as KOH. It is unnecessary to search therefore for an accompanying anion to explain the movement of potassium between muscle and blood.

The potassium content of muscles after 5 hours in Ringer's solution at low carbon dioxide tensions is roughly 7.31 m.-eq. per cent in table 4 (average of 3 only). This is 0.49 m.-eq. per cent less than the corresponding value in blood. Values in table 5 show that under these conditions the muscles in Ringer's solution have a carbon dioxide combining capacity of 9.1 volumes per cent as compared to 18.8 volumes per cent in blood. This is a difference of 9.7 volumes per cent or 0.43 m.-eq. per cent where the potassium loss is 0.49 m.-eq. per cent. While this comparison is not very accurate it suggests that in this case the potassium loss in Ringer's solution

is also chiefly due to diffusion of potassium as KOH to equalize the pH difference between the inside and the outside of the fibres. That this is not always true, however, was shown repeatedly in our previous study of muscles in Ringer's solution of varying pH (Fenn and Cobb, 1934).

Potassium intake from blood of high potassium content. It has been shown that frog muscles immersed in alkaline Ringer's solution with increased potassium content (Fenn and Cobb, 1934) will gain potassium. This is strong evidence in favor of a true membrane equilibrium between muscle and plasma with a membrane permeable to potassium ions, for in order to gain potassium this ion must move against the concentration gradient. On account of the importance of this finding and the relatively few cases in which we have observed it, we performed three experiments in which dry potassium chloride was added to blood in which sartorius muscles were immersed. The amount added was sufficient to increase the normal potassium content three-fold, i.e., from 0.4 to 1.22 m.-eq. per cent. In every case the potassium content of the muscles was increased after immersion as compared to the control muscle analyzed immediately after dissection. The amounts of potassium gained in this way in the three experiments were respectively 0.92, 1.33 and 0.92 m.-eq. per 100 grams of muscle. The change in weight of the muscles was negligible and the amount gained was greater than that needed to raise the concentration of potassium in the tissue spaces to the higher level or even to raise the concentration of potassium in all the muscle water to that level. The potassium was therefore taken in against the concentration gradient.

The intake of potassium as a result of increased concentration of potassium in Ringer's solution surrounding the muscle was found in our former paper (1934) to cause an increase in the bicarbonate content, indicating that some of it went in as KOH; but only about 9 per cent of the potassium diffusion could be accounted for in this way. Two similar experiments were performed in blood. Matched sartorius muscles were immersed, one in normal blood and the other in blood with the addition of approximately 1 m.-eq. of potassium per 100 grams of plasma. Both were equilibrated with 5 per cent carbon dioxide for 5 hours, and then analyzed for bicarbonate (in respirometers). The contents of the respirometers were later rinsed into crucibles and analyzed for potassium. The high-potassium muscles contained 1.35 and 1.05 m.-eq. per cent more potassium than the control muscle and only 0.18 and 0.21 m.-eq. per cent more bicarbonate respectively. This increased carbon dioxide capacity as a result of high potassium could of course be explained by phosphocreatine breakdown which is known to occur (Hegnauer, Fenn and Cobb, 1934) or it may be that all the potassium goes in as KOH most of which, however, is neutralized by lactic acid formation in excess of the phosphocreatine breakdown. In any event these experiments in blood agree closely with our former experiments in Ringer.

Absence of a chloride shift to muscle. Having obtained evidence for a potassium shift we next proceeded to look for a chloride shift from plasma to muscles. For this purpose we proceeded in the same way but analyzed the muscles for chloride content by the Westfall micro method. Other thigh muscles were removed from the same animal at dissection and analyzed for initial chloride by the Van Slyke method. The blood was also

TABLE 6

Chloride gain by muscles in 1 per cent and 9.3 per cent carbon dioxide in blood

PLASMA CHLORIDE		MUSCLE CHLORIDE			CHLORIDE SPACE			Δ WEIGHT	
			After soaking			After soaking			
1 per cent CO_2	9.3 per cent CO_2	Initial	1 per cent CO_2	9.3 per cent CO_2	Initial	1 per cent CO_2	9.3 per cent CO_2	1 per cent	9.3 per cent
m.-eq.%	*m.-eq.%*	*m.-eq.%*	*m.-eq.%*†	*m.-eq.%*†	%	%	%	%	%
6.75	6.26	0.49	1.60	2.69	7.5	23.7	43.0	−8.6	−9.2
		0.68	1.63	1.45	9.5	24.2	23.2	+2.5	+0.8
		0.75	1.62	1.91	11.5	24.0	30.5	−3.6	−4.3
6.93	6.57	0.71	1.00	1.76	10.5	14.4	26.8	−9.5	−5.3
		0.86	1.06	1.46	12.7	15.3	22.2	−4.6	−4.8
7.19	7.19*	0.78	1.69	1.16	10.9	23.5	16.1	+4.3	+11.5
7.72	7.72	0.83	1.79	1.06	10.7	23.2	13.7	+0.9	−5.5
(8.11)	(5.96)	1.30	2.51	2.07	16.0	30.9	25.5	−5.1	−6.5
7.72	7.63	0.95	2.46	2.44	12.4	31.9	32.0	−1.8	−0.9
Average 7.26	7.07	0.82	1.71	1.78	11.3	23.5	25.9	−2.8	−2.7

The 1 per cent CO_2 varied from 0.9 to 1.15 per cent in different experiments. The plasma was analyzed after the muscles were removed. The average of the 1 per cent and 9.3 per cent values was used in calculating the initial chloride space. Figures for plasma chloride in parentheses are not included in the average. The 1 per cent CO_2 value for plasma chloride was used in calculations.

* 23 per cent CO_2 instead of 9.3 per cent.

† Per 100 grams weight after soaking.

centrifuged immediately after the muscles were removed and the plasma was analyzed in 0.217 cc. samples for chloride by Patterson's modification of the open Carius method. The results are included in table 6. The averages of nine experiments show that the chloride content after soaking in 1 per cent carbon dioxide was 1.71 m.-eq. per cent as compared to 1.78 in 9.3 per cent carbon dioxide. This close agreement gives a false idea of the uniformity of the experiments, however, for in approximately half

the cases the chloride content was greater in the low carbon dioxide tension while the reverse was the case in the other half, and the differences were rather large.

The last three columns of table 6 show the magnitude of the chloride spaces, or in other words, that fraction of the muscle which must be assumed to contain chloride in the same concentration as that in the external solution. It is calculated by dividing the chloride content of the muscle by that of the solution (cf. Fenn, Cobb, and Marsh, 1934). The averages show that the initial chloride space is only 11.3 per cent of the muscle whereas after soaking it is 23.5 and 25.9 per cent in the 1 per cent and 9.3 per cent carbon dioxide tensions, respectively. There is, therefore, even in the frog's own blood equilibrated with carbon dioxide a large increase in the chloride space, and the chloride content practically doubles. This gain in chloride appears to be independent of carbon dioxide tension as far as can be ascertained by this method. The variations, however, are rather large so that the results do not entirely exclude a possible small chloride shift, which is entirely overbalanced by other more important factors.

We have been unable to discover or control the factor responsible for this chloride gain in blood. Chilling the frog previous to dissection to reduce the stimulation resulting from crushing the brain had no effect. Doubling the amount of heparin causes only a slight increase in the chloride gained which might have been experimental error. Injuring the muscle during dissection by laying it on the frog skin so as to cause a contracture produced likewise only a small increase in the chloride content which was no larger than the variations observed in table 6.

It may be observed also in table 6 that the chloride content of the plasma is slightly less at the high carbon dioxide tension. This can be explained by the well known chloride shift to the corpuscles. There is no apparent correlation between the percentage changes in weight and the chloride gains in table 6. On the average the loss in weight was equal in both high and low carbon dioxide tensions but this varied markedly from one muscle to the next, some gaining weight and some losing weight.

A similar absence of a clean-cut chloride shift was obtained in three earlier experiments in which variations of pH of the solutions were obtained by phosphate buffers, the solutions being equilibrated with pure oxygen rather than with CO_2-O_2 mixtures. Two of these experiments showed more chloride in the muscle soaked in the more alkaline solution (3.88 and 3.38 m.-eq. per cent in the alkaline muscle compared to 3.78 and 3.29, respectively, in the acid muscles) while the third experiment involving more muscles showed the reverse (3.35 m.-eq. per cent in the acid and 2.76 in the alkaline muscles).

Christy (1927) has described a chloride shift to the tissues in exercise,

and Essen, Kanders and Porges (1932) have described cases in which chloride varies inversely as the alveolar carbon dioxide tension (and the blood HCO_3). These observations, however, are to be explained probably as shifts of NaCl and not as true "chloride shift" where chloride exchanges with another anion. Thus, in exercise Ewig and Wiener (1928) as well as Dill, Talbot and Edwards (1930) have concluded that both sodium and chloride enter the tissues from the blood with a corresponding amount of water.

Discussion. While these experiments show that a potassium shift can occur between plasma and muscle as a result of increased carbon dioxide tension they do not prove that such a shift will actually occur in the human body. It is quite possible that the pH changes which are produced by a rise in carbon dioxide tension will be neutralized by shifts of undissociated lactic acid, a decrease in the lactic acid level in the muscle, or an increased breakdown of phosphocreatine before any potassium can shift. Absence of such a potassium shift in man has actually been reported in experiments by Gollwitzer-Meier (1925).

The chief importance of these experiments is the further evidence which they afford in favor of the conception that a muscle is a potassium-permeable but sodium-impermeable and anion-impermeable structure. Contrary to this view it is now frequently assumed (Peters and Van Slyke, (1931) for example) in the absence of evidence to the contrary, that the tissue cells are comparable in their permeability properties to the red blood corpuscle. All the known facts are equally well explained, so far as we can ascertain, on the theory that potassium is immobilized in the tissue because it is combined with indiffusible anions.

SUMMARY

Frog sartorius muscles, during immersion in frog blood *in vitro*, usually lose some potassium. If the control muscles are immersed a short time in blood to permit loss of potassium from injured fibres and if the blood is equilibrated with a high carbon dioxide tension then the immersed muscles usually gain potassium during immersion. If two matched sartorius muscles are immersed in frog blood one at a high and the other at a low carbon dioxide tension, the muscle in the higher carbon dioxide tension shows the higher potassium content. Measurements of the carbon dioxide combining capacity show that where the potassium content is high the bicarbonate content is high also by an approximately equivalent amount. Muscles immersed in frog blood containing an increased amount. of potassium and equilibrated with 5 per cent carbon dioxide show a gain in potassium representing a diffusion of potassium into the muscle against a concentration gradient. No evidence for a chloride shift to the muscle could be found. In frog blood isolated sartorius muscles gain consid-

erably in chloride content and their extracellular spaces are increased in volume, although this change is less in blood than in Ringer's solution.

It is concluded that an increase in carbon dioxide tension tends to cause potassium to shift from plasma to muscles but the same increase in carbon dioxide would cause the potassium to move from the muscles to Ringer's solution because the muscle is intermediate between Ringer's solution and blood in its buffering capacity.

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THE KINETICS OF THE ELIMINATION OF THE DYE WATER BLUE FROM DOG PLASMA AFTER INTRAVENOUS INJECTION

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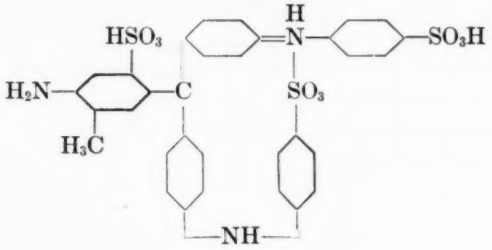
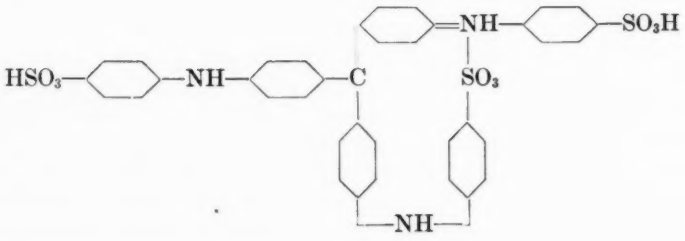
Received for publication February 5, 1935

Water blue is a colloidal acid dye formed by sulphonation of the insoluble aniline blue. The dye is probably a mixture containing the di- and trisulphonates of diphenylrosaniline and triphenyl pararosaniline. Two possible components according to Conn (1) are (I) the trisulphonate of triphenyl pararosaniline and (II) the trisulphonate of diphenyl rosaniline, the respective formulas being as follows:



I



II

This dye is also called cotton blue, China blue and water soluble aniline blue. Its physiological properties are its relative non-toxicity in concentrations sufficient to cause intense coloration of living tissues in man (2)

and dogs (3), and its ability to remain in plasma after intravenous injection longer than other known dyes (4). Except for a single excretion curve of Wittgenstein and Krebs (4) and two observations of Dawnson, Evans and Whipple (3) made in the hour following injection, there are no quantitative excretory data on this dye.

In order to measure relative plasma volume a dye must be used which is not eliminated to any appreciable extent from the blood stream in the interval during which changes in blood volume are measured. Due to the slow elimination of water blue from the plasma, this dye seemed to fulfill the necessary requirements. In order to test whether or not this dye is suitable for measurements of relative plasma volume accurate excretion data must be known. In the present study elimination rates of the dye from plasma of dogs after intravenous injection have been measured over twelve day intervals. In addition the elimination rate has been measured during a four hour interval several days after the injection. As will be mentioned later these excretion data are confirmatory of the theories of Smith (5) (6), in regard to the removal of dye from plasma by phagocytosis.

METHOD. Water blue (Kahlbaum 3B) was dissolved in sterile saline and injected intravenously into normal unanesthetized healthy adult dogs varying in weight from 10 to 25 kilos. The amount injected was 40 mgm. per milliliter per kilo. Within 3 to 6 minutes after injection the first sample of blood, about 8 ml., was withdrawn from the femoral artery or jugular vein and discharged into a calibrated 15 ml. centrifuge tube containing 1 ml. of 1.6 per cent sodium oxalate. After centrifuging, the cell volume and diluted plasma volume were read. A sample of plasma was removed and mixed with an equal volume of 0.2 N HCl, this developing the blue color to maximum intensity. This first sample of plasma was used as a standard and its relative concentration designated as 100.

Blood samples were obtained at various time intervals after the injection, usually three in the first hour after injection and three more during the next 24 hours, then one sample per day for the following eleven days. In computing relative concentrations of the dye in plasma it is, of course, necessary to consider the hematocrit readings. A simple calculation will show that the relative concentration of the second sample of plasma, C_2, in terms of the concentration of the first sample, $C_1 = 100$, is

$$C_2 = 100 \frac{1_1}{1_2} \frac{P_1(P_{2+1})}{P_2(P_{1+1})}$$

where 1_1 and 1_2 are the readings of the Duboscq colorimeter of samples (1) and (2). P_1 and P_2 are the corresponding diluted plasma volumes (diluted with oxalate) of samples (1) and (2). A similar relation holds for subsequent samples.

Turbidity and phenol extraction. In order to develop the intense blue

color acid must be added to plasma. In some cases, usually very few, a turbidity develops on addition of the acid. The turbidity is quite irregular and increases on standing and is probably due to denatured protein. After numerous experiments with HCl and acetic acid of various strength it was found that 0.2 N HCl added in an amount equal to that of the plasma produced maximum color intensity with least turbidity. Where turbidity is produced it can be eliminated by either of the two following procedures.

(1) The use of color filters with a Duboscq colorimeter or the use of a spectrophotometer. The following absorption curve, figure 1, shows the

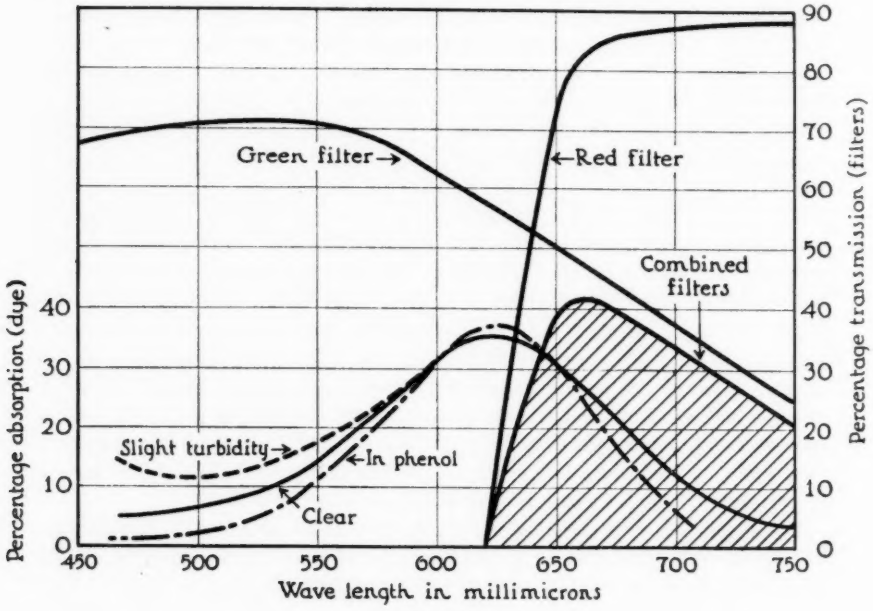


Fig. 1

effect of turbidity on the light absorption of the acidulated plasma containing water blue. Two samples of dyed plasma were chosen, having absorption maxima of approximately the same value, one solution being clear and the other slightly turbid. The effect of turbidity is to increase the light absorption in the blue-violet region of the spectrum. This causes a change in shade of the color, the turbid solution having a reddish tinge, which makes matching in the colorimeter inaccurate. It is to be observed, however, that absorption due to turbidity is much less in the red region of the spectrum. The turbidity error can, therefore, be reduced to an almost negligible amount by using color filters over the eyepiece of the colorimeter,

which permit red light to be filtered through. A suitable light filter combination consists of the Corning filters "H. R." red 3.95 mm. and "heat absorbing" green 2.95 mm. in thickness. The transmitted light lies in a wave length region as given in figure 1.

If the spectrophotometer is to be used rather than the Duboscq colorimeter the prism is set to transmit light having a wave-length $\lambda = 620$ mμ. The turbid solution acidulated plasma is divided into two equal parts, and to one part is added 1 drop of concentrated Na_2SO_3. This decolorizes the water blue but the turbidity remains. This decolorized sample is then placed in one beam of the spectrophotometer and the colored sample in the other. Measured in this way the turbidity is compensated and the observed absorption is due entirely to the dye.

(2) Attempts were made to precipitate protein with colorless reagents. In all cases where the proteins were completely precipitated the dye was also precipitated. On a suggestion from Mr. H. P. Lundgren it was found that phenol would completely extract the dye from plasma forming a clear non-turbid phenol phase although the plasma itself showed considerable turbidity. For turbid solutions the procedure finally used was to add 5 cc. of phenol saturated with water to the acidulated turbid plasma, shake and centrifuge, jacketing the centrifuge tubes with ice water. After ten minutes' centrifugation the phenol would be a few degrees below room temperature and would contain *all* of the dye. The phenol phase was immediately removed. If the centrifuge tube is not cooled during centrifuging, its temperature will rise a few degrees. On removing the phenol phase and allowing the temperature to drop to room temperature small droplets of water separate, causing a turbidity due to water in the phenol solution. This is prevented by cooling during centrifuging. The error due to cooling the phenol a few degrees below room temperature when separating the two phases, insofar as the phase composition is concerned, is seen to be negligible from the phenol-water solubility curves. Furthermore, all measurements are comparative and are made under identical conditions; hence a slight phase volume change of the phenol phase due to cooling is the same for each experiment.

EXPERIMENTAL RESULTS. I. *The elimination over a twelve day interval.* Figure 2 gives in the form of a graph the relative dye concentration in the plasma of six dogs at various time intervals after intravenous injection. In five cases Kahlbaum's dye "Wasser Blau 3 B" was used. In one case Merck's water soluble Aniline Blue 2 B was used as indicated. It is to be noted that there is a rapid drop in the first few hours after injection followed by a slower elimination lasting 3 to 4 days, then an excretion for the next 7 or 8 days at an approximately linear rate. The resemblance of this excretion data to that of Smith (6) is striking. The general form of the excretion curve of Wasser Blau is similar to that of brilliant vital red (Smith, 6),

differing only in that Wasser Blau after the rapid initial fall disappears more slowly.

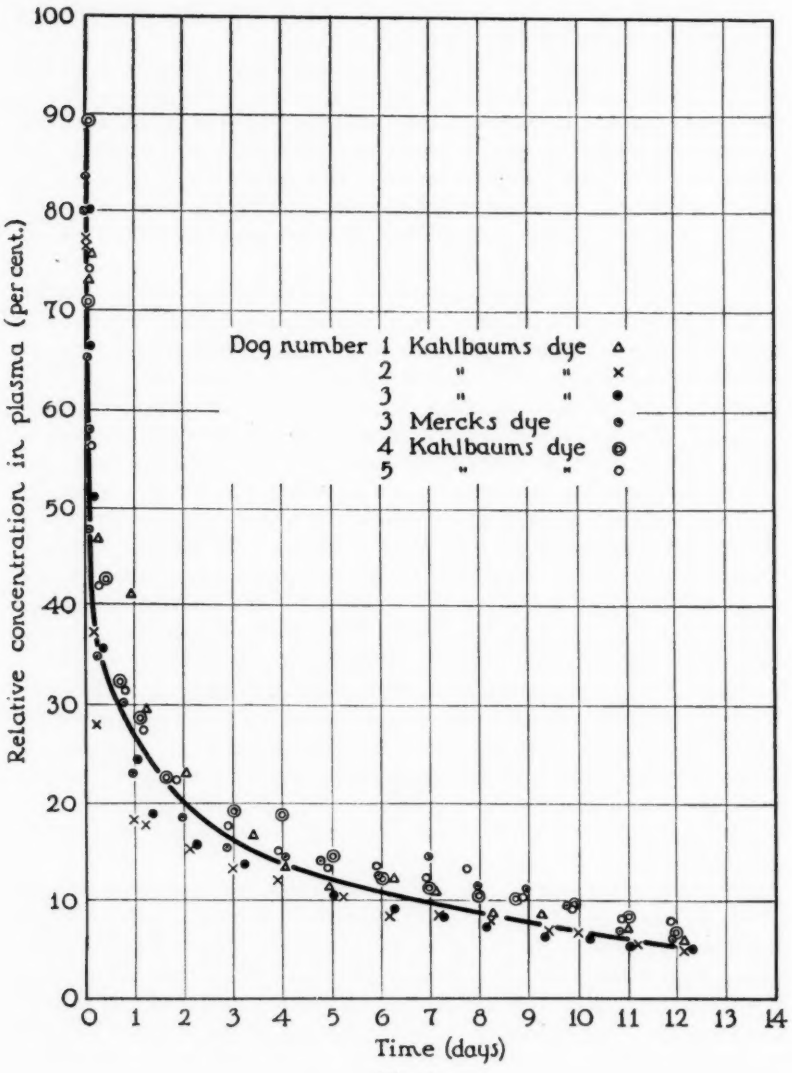


Fig. 2

Smith (5), (6) believes that the dye immediately after injection is removed and stored in the reticulo-endothelial system. The removal is

rapid at first but gradually approaches an equilibrium value where the dye of the cells is in equilibrium with that of the plasma. Superimposed upon this removal into the cells is a slower elimination from the plasma, mainly through the bile. If it be assumed that the plasma dye leaves the plasma to reach finally an equilibrium distribution between cells (reticulo-endothelial) and plasma, then the usual laws of diffusion into a second phase tending to a partition equilibrium may be used. The differentiation equation is

$$\frac{dC_1}{dt} = k(nC_1 - C_2)$$

where k = diffusion coefficient, C_1 is plasma concentration, C_2 is cell concentration and n is the equilibrium partition distribution coefficient $n\frac{C_1}{C_2}$ at equilibrium. A solution of this equation gives the usual exponential relation

$$C_t = C_0\, e^{-mt} + D$$

where C_0 is the initial concentration of the dye in the plasma and C_t is the concentration at any time t. If $ln\ C_t$ is plotted against t on semi-logarithmic paper a straight line should result. When this plot is made it is found that a straight line does result for the excretion during the first two hours, but after this there is a sudden change of slope and another straight line with a different slope represents the excretion for the next 48 to 72 hours. After this the rate of elimination is almost constant. The excretion can therefore be well represented by the equation

$$C_t = A_{\bullet}^{-at} + B_{\bullet}^{-bt} - vt + D$$

where C_t is the relative plasma concentration of the dye at any time t and A, B, a, b, v and D are constants for each curve independent of C and t. This indicates that there are two processes whereby the dye diffuses from the plasma to a partition equilibrium, namely, a rapid diffusion which is complete in two hours, constants A and a, and a diffusion process which takes place more slowly, constants B and b, this latter process reaching equilibrium after 2 to 3 days. Superimposed on this there is continuous linear elimination at a constant rate v and not proportional to concentration during the time limits of the experiments. A constant elimination independent of concentration shows that this last process is not a simple diffusion. It may be due to activities of the liver cells causing removal of the dye by way of the bile, as suggested by Smith, who observed that such a slow removal of brilliant vital red was not dependent on concentration. In table 1 there is given the curve constants determined for the six dogs

(with averages for the five dogs) in which the Kahlbaum dye was used. From the average values of the constants the continuous curve of figure 2 was drawn, showing a good agreement between the experimental values and the theoretical curve. This agreement lends weight to the excretion theories of Smith (6), his postulates being contained in the above mathematical equation.

TABLE 1

Constants of the curve

$$C_t = A_0 e^{-at} + B_0 e^{-bt} - vt + D$$

where C_t is the dye concentration in plasma at a time t after intravenous injection of the dye.

DOG NUMBER	WEIGHT	V UNITS/DAY	D UNITS	A_0 UNITS	a (MIN.)$^{-1}$	B_0 UNITS	b (MIN.)$^{-1}$
	kgm.						
1	21.3	0.83	16.0	47	9.5×10^{-3}	37	5.3×10^{-4}
2	19.0	0.66	12.5	77.5	7.4	10	2.6×10^{-4}
3*	13.6	1.05	19.0	51	12.5	30	4.0×10^{-4}
3		0.80	14.2	61.5	9.9×10^{-3}	24	5.3×10^{-4}
4	18.4	0.96	18.5	57.5	12.2×10^{-3}	24	3.9×10^{-4}
5	20.5	0.86	17.5	56.5	9.5×10^{-3}	26	3.8×10^{-4}
Average.....		0.82	15.7	60	9.7×10^{-3}	24	4.2×10^{-4}

* Merck's dye used.

TABLE 2

Relative hemoglobin and dye concentration in plasma of normal unanesthetized dogs 6 to 7 days after intravenous injection of 40 mgm. water blue per kilo

DOG NUMBER	DAYS AFTER INJECTION	TIME ELAPSED (HOURS)									
		0		1		2		3		4	
		Dye	Hb	Dye	Hb	Dye	Hb	Dye	Hb	Dye	Hb
1	7	100	100	96.5	90.9	95.0	92.9	87.5	81.1	85.4	80.5
2	6	100	100	96.2	93.2	92.5	89.9	93.4	92.0	89.0	86.3
4	7	100	100	97.1	95.0	91.7	95.0	89.6	95.0	90.9	93.8

II. *Dye excretion over a four-hour interval several days after intravenous injection.* In order to measure more accurately the slow elimination several days after injection a four-hour interval was chosen with blood samples drawn from the femoral artery every hour. The results are given in table 2. In every case the Kahlbaum 3 B dye was used.

From the average excretion curve drawn from the averages of the constants, seven days after injection the relative concentration would be 10.

The average rate of excretion per day is $v = 0.82$ unit per day. During a four hour interval there would be a theoretical drop in concentration due to this slow elimination mechanism from 10 to 9.833, i.e., a drop in concentration in per cent from 100 to 98.3. Actually the measured decrease was much greater than this, amounting to a relative decrease of 10 to 15 per cent instead of the theoretical 2 per cent. Over a four hour interval changes in plasma volume would also cause a change in dye concentration. Relative hemoglobin determinations were made at the same time that the dye concentration was determined. In every case, there was a decrease in hemoglobin for the second, third and fourth, etc., samples; and the decrease in hemoglobin paralleled within the limits of error the decrease in dye concentration. A similar result is to be noted in experiments of Chanutin, Smith and Mendel (7). In a series of hourly samples of hemoglobin drawn from normal unanesthetized dogs there was in 30 cases out of 31 always a decrease (or no change) in the hemoglobin concentration. These results together with ours indicate that successive samples drawn an hour apart give hemoglobin values always lower than the initial sample. The explanation of this decrease in hemoglobin is apparently unknown. It must be taken into consideration in studying changes in the chemical composition of blood over an experimental period when normal unanesthetized animals are used. This hemoglobin change together with the change in dye concentration indicates a dilution of the plasma.

SUMMARY

The elimination of the triphenyl methane dye water blue from dog plasma after intravenous injection has been studied over a 12 day interval. The decrease in concentration is rapid during the first two hours and follows an exponential curve. During the next 48 to 72 hours the elimination follows a slower exponential curve and during the last 7 to 8 days the dye is removed at an approximate constant rate. The concentration plotted against time fits very closely the curve

$$C = Ae^{-at} + Be^{-bt} - vt + D$$

where C is the concentration at the time t and A, a, B, b, v and D are constants obtainable from the curve and derived theoretically from the theory of Smith (5, 6) in regard to dye removal from plasma.

Six to seven days after the injection of water blue (40 mgm. per kilo per cc. sterile saline) the dye is eliminated slowly at a rate of about 2 per cent over a four hour interval. Repeated sampling at hourly intervals causes an apparent plasma dilution in normal unanesthetized dogs whereby the hemoglobin concentration and dye concentration decrease, the decrease in dye paralleling the decrease in hemoglobin.

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TIME FOR DEVELOPMENT OF INCAPACITY TO WORK IN ADRENALECTOMIZED RATS

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Received for publication February 5, 1935

The demonstration (2) that the intact gastrocnemius muscle of normal anesthetized rats can sustain a high rate of work output for periods of ten days and longer provides an indicator for studying the development of adrenal insufficiency after removal of the adrenal bodies. It was proposed to begin stimulating the muscle of rats immediately following adrenalectomy and to observe the time for development of "fatigue." A preliminary study (3) of this and other problems has been published. The present experiments involve some changes in method.

METHOD. The technique for preparing an animal and the apparatus for stimulating the muscle and recording work have been described (2) in detail. The rats were first selected for resistance to anesthesia by the subcutaneous injection of a standard dose of sodium luminal. The 90 per cent which survived the test were allowed a minimum of two weeks for recovery. For the experimental anesthetization those to be operated received 70 per cent as much luminal as the unoperated controls with ether administered to obtain surgical anesthesia during the operation.

Both adrenals were removed in a single stage aseptic operation by the usual dorso-lateral approach with complete avoidance of postoperative hemorrhage and infection. Six animals were worked simultaneously, the gastrocnemius of each being connected in series and stimulated to lift a 100 gram weight three times per second. Stimulation began within one hour after operation and continued until the death of the animal or to a maximum of 120 hours. At eight hour intervals the experimenter recorded the minute rate and total revolutions from Veeder counters attached to automatic work adders, administered subcutaneously 0.5 cc. distilled water per 100 grams body weight, and injected sufficient luminal to keep the animal immobilized and unresponsive to the faradic shock. Temperature and humidity were constant.

RESULTS. 1. Table 1 summarizes the performance of bilaterally adrenalectomized rats, controls with a kidney and the adrenal of the opposite side removed, and normal animals. The significant comparisons include the rate of work per minute at the end of each eight hour period

TABLE 1

Work records of adrenalectomized, control operated, and normal male rats

GROUP		AGE	WEIGHT	RATE‡ PER MINUTE AT 8 HOUR INTERVALS										HOURS OF WORK	TOTAL REVOLUTIONS
				0	8	16	24	32	40	48	56	64	72		
		days	*grams*												
I	n*	94	214	27	28	26	19	17	17	16	18	18	14	120	132,900
	k**	94	214	25	25	25	22	21	22	20	19	19	14	120	137,080
	b†	94	214	26	23	11	—	—	—	—	—	—	—	19	20,890
II	n	60	225	32	26	21	20	21	19	20	21	22	22	120	157,440
	k	60	220	22	27	25	8	7	4	1	1	1	2	120	45,912
	b	60	239	26	24	20	3	—	—	—	—	—	—	27	26,040
III	n	480	312	38	31	22	12	15	21	25	27	28	22	120	168,060
	k	480	316	38	29	22	14	16	20	23	24	23	21	120	156,590
	b	480	290	34	16	—	—	—	—	—	—	—	—	10	14,390
	b	480	283	27	23	—	—	—	—	—	—	—	—	10	14,940
IV	n	480	370	36	37	30	23	23	21	22	30	30	28	120	196,410
	k	480	361	37	46	34	21	22	26	28	30	30	29	120	205,090
	b	480	375	34	38	17	5	—	—	—	—	—	—	25	38,990
V	n	83	249	29	33	26	23	23	23	22	20	16	19	120	152,920
	k	92	233	27	26	25	16	13	12	12	10	8	8	120	87,640
	b	94	264	38	34	4	—	—	—	—	—	—	—	23	29,620
VI	n	62	192	30	30	25	13	+0	+0	+0	2	4	6	120	63,355
	k	62	198	26	27	26	24	22	26	23	22	20	19	120	150,410
	b	62	201	20	—	—	—	—	—	—	—	—	—	7	4,660
	b	62	195	24	—	—	—	—	—	—	—	—	—	6	15,250
VII	n	93	200	28	22	21	20	21	19	21	23	22	20	120	125,070
	k	93	200	28	23	19	13	14	13	15	15	15	14	120	83,420
	b	93	210	29	19	14	10	—	—	—	—	—	—	32	24,790
VIII	n	74	195	34	26	28	21	14	15	12	15	3	+0	120	76,610
	k	74	204	29	28	24	24	24	22	24	24	23	22	120	159,240
	b	74	207	29	25	20	4	—	—	—	—	—	—	28	28,580
IX	n	80	197	30	31	27	23	22	21	22	25	26	24	120	170,630
	k	80	197	27	23	20	9	4	12	+0	+0	+0	+0	120	33,575
	b	80	197	36	29	17	—	—	—	—	—	—	—	23	28,575
X	n	?	316	45	42	38	30	32	28	28	30	28	29	120	215,990
	k	?	318	42	40	36	28	29	28	28	30	28	30	120	207,740
	b	?	322	45	20	7	—	—	—	—	—	—	—	30	12,590

* Normal.

** Left adrenal and right kidney removed.

† Bilaterally adrenalectomized.

‡ Recorder revolutions each approximating 400 gram-centimeters work.

up to 72 hours, total hours of work, and total work accomplished before "fatigue" or a maximum of 120 hours.

It is apparent that the capacity for sustaining work among animals subjected to severe control operations compares favorably with that of unoperated animals. In contrast the completely adrenalectomized animals work normally for only a short time before developing a breakdown of muscular responsiveness.

2. Among a total of 45 bilaterally adrenalectomized animals which began work immediately after operation 6.6 per cent exhibited breakdown by the 8 hour period, 53 per cent by 16 hours, 78 per cent by 24 hours, 96 per cent by 32 hours, and no animal exceeded 36 hours. When the opposite leg was stimulated immediately after the breakdown, it contracted normally at first but lost its capacity for response within a few minutes. The animals succumbed on the average 2½ hours after cessation of muscular contractions.

3. That anesthesia shortens the survival period of adrenalectomized animals is well known as is also the observation (1) that exercise hastens the manifestation of adrenal insufficiency. To test these two conditions one each of 20 like-sex litter-mate pairs matched for weight was subjected to work of the gastrocnemius muscle, while the control animal was treated in an identical manner except for the omission of the weighting and stimulating of the muscle. The "work" animals survived from 10 to 40 hours with an average of 26 hours, while the "no-work" group ranged from 10 to 211 hours with an average of 64 hours. In only one instance did a "no-work" animal die before its "work" control.

4. Evidence of the demands of continuous muscular contraction and of anesthesia upon the adrenalectomized organism is apparent from comparing results, 1, 2 and 3 with the survival under optimal living cage conditions of 42 bilaterally adrenalectomized rats which lived from 7 to 32 days with an average of 20 days.

5. Administration of adrenal cortex extracts[1] not only effectively prevents the breakdown of anesthetized working adrenalectomized rats but also brings about recovery once "fatigue" has occurred. This problem is the subject of a detailed investigation and will be treated in a later report.

The results of these studies substantiate those of a preliminary report (3).

Discussion. The rat has not been universally accepted as a suitable animal for studies of adrenal insufficiency. The striking lack of uniformity among results of such studies on these animals is probably due to individual or strain differences in amount of accessory cortical tissue, differences in diet, particularly in amount of sodium chloride included, and incomplete removal of the gland. In these experiments diet was constant, and it is

[1] Efficacious extracts from the adrenal cortex were made available to us through the kindness of Dr. E. C. Kendall, The Mayo Foundation and Dr. Oliver Kamm, Parke, Davis & Company.

manifest that accessory tissue, although a likely factor producing individual differences in work and survival has not prevented the demonstration of adrenal insufficiency in the rat either in the optimal living cage situation or under conditions of stress. As to technique the animals were prepared by one of us who had already the experience of performing several thousand adrenalectomies.

It is necessary to examine the possibility that operative shock rather than glandular insufficiency accounts for the breakdown of working adrenalectomized animals. Several considerations, however, point to the absence of the adrenals themselves as the essential factor. Operative shock is difficult to produce in the rat. The removal of one adrenal and the opposite kidney as in the control operations should provide a trauma at least equivalent to that from bilateral adrenalectomy, but the performance of the operated controls is like that of the normals and clearly differentiated from that of the experimentals. Moreover, the breakdown occurs several hours after operation, much later than operative shock would be expected. Finally, the fact that the adrenalectomized animals can be sustained with cortin provides strong evidence that one is concerned with an insufficiency of this hormone.

The loss of capacity to work under the experimental conditions cannot properly be regarded as fatigue although the performance of work hastens the development of the deficit. When the unworked gastrocnemius is stimulated after "fatigue" of the working leg, it contracts normally but "fatigues" within a few minutes. This, together with the observation that body temperature is lowered and that a general cyanosis appears supports the authors' view that the condition is best described as an "adrenal shock" and that the decrement in intensity of contraction reflects the onset of shock.

In addition to work and anesthesia several other factors probably contribute to the final breakdown of the experimental animals. Distilled water was injected subcutaneously, an unphysiological procedure but one well tolerated by normal rats and even adrenalectomized animals in early stages of insufficiency. When work output has dropped to a low level, the injection of water may be followed by the abolishment of muscular contractions within very few minutes, and possible death. The marked edema which develops in the extremities of the working adrenalectomized animal may provide toxic substances. But while certain factors may contribute to the differences observed between the work capacity of normal and adrenalectomized animals, the results unequivocally require absence of the adrenal bodies as the necessary condition.

SUMMARY

Bilaterally adrenalectomized rats in optimal living quarters survived from 7 to 32 days. Similar animals anesthetized with sodium luminal and

receiving water by subcutaneous injection lived from 10 to 211 hours or an average of 64 hours. Stimulating the weighted muscle of animals matched with the previous group for every other experimental condition lowered the average survival period to 26 hours with a range from 10 to 40 hours.

Adrenalectomized, anesthetized animals forced to work immediately after operation performed normally at first but within a maximum of 36 hours developed a "fatigue" which may be regarded as adrenal shock. Operated controls worked like normal animals. The "fatigue" of the adrenalectomized animals may be prevented or reversed by administration of adrenal cortex extracts.

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THE INACTIVATION OF HISTAMINE IN PERFUSED ORGANS

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Received for publication February 23, 1935

A series of investigations in this laboratory has been devoted to the site of inactivation of a number of alkaloids with special reference to the part played by the liver in their elimination from the blood of the dog. Priestley, Markowitz and Mann (1931) demonstrated that the liver was more effective in removing strychnine than other tissues of the body. Biebl, Essex and Mann (1932) found the same to be true in the case of nicotine. The liver did not appear to be more capable of eliminating ephedrine from the blood than the other tissues studied by Thorp, Essex, and Mann (1933).

The inactivation of histamine was the subject of an extensive study by Best and McHenry (1930). The perfusion experiments carried out by these workers were done by means of the Dale-Schuster system of diaphragm pumps.

It was thought that an investigation of the inactivation of histamine in a manner similar to that of previous studies reported from this laboratory might prove of value. It should be stated here that with the exception of the results with the perfused liver, our findings are in agreement with the perfusion experiments of Best and McHenry.

METHODS AND RESULTS. As in previous studies, the isolated organs (hind limbs, liver, intestines, and kidneys) were perfused by the use of the Starling heart-lung preparation. Defibrinated blood, containing a small amount of heparin, was used for the perfusing medium. About 1000 cc. of blood were used in each experiment.

The degree of inactivation of histamine was determined by the effect of successive samples of the perfusing blood on the blood pressure of dogs, weighing from 6 to 8 kgm., which were anesthetized with sodium amytal.

In each experiment the histamine was added to the venous reservoir of the heart-lung preparation, and after thorough stirring a control sample was removed. The doses of histamine employed varied from 20 to 100 mgm. Two experiments were sufficient to indicate the inadvisability of doses as great as 100 mgm. The heart-lung preparation survived doses of this size but maximal effects were obtained on the blood pressure of the test animal when 10 cc. of blood from the heart-lung-liver preparation were

given. It was therefore difficult to determine whether inactivation had occurred even though the blood containing histamine had been perfused through the heart-lung-liver preparation for nearly three hours. Consequently, doses of from 20 to 50 mgm. were used in subsequent experiments.

Heart-lung preparation. It was first necessary to determine the ability of the heart and lungs to inactivate histamine. In two experiments 20 and 30 mgm. of the drug, respectively, were used. After three hours' perfusion such a small amount of the amine had been removed that the use of the heart-lung preparation for perfusing other organs did not appear open to serious objection.

Heart-lung-hind limbs. The hind limbs were prepared for perfusion according to the method employed by Thorp, Essex, and Mann, which briefly described consists of placing the hind limbs of another dog, weighing from 6 to 8 kgm., in the heart-lung circuit by cannulating the abdominal aorta and vena cava in the lower portion of the abdomen, the remainder of the animal being eliminated by appropriate ligatures and sectioning. By this method the limbs were kept in excellent condition and responded to electrical stimulation in a normal manner at the end of the experiment. In such a preparation a dose as small as 25 mgm. was not inactivated after perfusion for one hour and forty-six minutes. There was, however, in three experiments a much greater reduction of histamine than occurred with the heart-lung preparation alone.

Intestine. The intestine was prepared for perfusion as follows: 1, the colon was sectioned anterior to the inferior mesenteric artery; 2, the duodenum was sectioned in the region of the tail of the pancreas, and 3, the mesentery was cut and the intestines were freed except in the region of the superior mesenteric artery and portal vein, which were then cannulated. After this the intestines were placed in a saline bath which was kept heated at body temperature. As soon as possible after removal, the inferior mesenteric artery was placed in the arterial circuit of the heart-lung preparation and perfusion was begun. By this technic the intestines were without arterial blood not longer than two minutes. After the preparation was completed, 25 mgm. of histamine were added to the venous reservoir and the same procedure as was employed in the other experiments was followed. As indicated by the blood pressure of the test animal, all of the histamine had not disappeared after an hour's perfusion. The results of two satisfactory experiments indicated that the perfused intestine does not inactivate histamine more rapidly than the perfused hind limbs.

Liver. The liver was prepared for perfusion by a method that permits an uninterrupted supply of arterial blood in the hepatic artery. After the heart-lung preparation had been made, the liver of another dog weighing from 6 to 8 kgm. was prepared for perfusion. This was accomplished by isolating the hepatic artery, around the proximal portion of which a loose

ligature was placed. The gastroduodenal branch of the hepatic artery was ligated, cannulated, and placed in the circuit of the heart-lung preparation. At the same time the ligature about the hepatic artery was tied and the artery sectioned. The portal vein was cannulated and perfused from the venous reservoir. The common bile duct was ligated, and the liver was removed in the usual manner and placed in a container from which an outlet carried the blood from the liver to the venous reservoir of the heart-lung preparation.

As compared with the hind limbs, the perfused liver eliminated histamine from the blood decidedly more rapidly. In a typical experiment 30 mgm. of the amine were eliminated in one hour and forty-five minutes. In another experiment 20 mgm. of histamine had practically disappeared in forty-five minutes.

Kidneys. Since the findings of Best and McHenry that the kidneys are more effective in eliminating histamine than the other organs perfused, it seemed particularly desirable that optimal conditions accompany the perfusing of these organs. The following method was used, which insures a minimum of disturbance of the kidneys in their preparation. As in the preparations of Best and McHenry, all branches of the aorta were ligated and sectioned, from the level of the superior mesenteric artery to a point posterior to the internal spermatic artery, at which point the aorta and vena cava were cannulated. A loose ligature was placed around the aorta and vena cava just distal to the superior mesenteric artery. The cannula in the aorta was connected with the arterial side of the heart-lung preparation. When this was completed, the loose ligatures about the aorta and vena cava were tied and, simultaneously, the clamps were removed from the aorta and vena cava, thus allowing the kidneys to be perfused at a blood pressure within physiologic limits. By this means the kidneys were never without arterial blood. By leaving the kidneys in situ, the trauma incident to removing them from the body was avoided, thus affording a more nearly normal preparation. The results obtained with such preparations are in striking contrast to those obtained with the other preparations described. Practically all of 25 mgm. of histamine was removed from the blood in three instances within fifteen minutes after the amine was added to the venous reservoir. These experiments amply confirm those of Best and McHenry and furnish additional proof of the ability of the kidneys to eliminate histamine from the blood.

SUMMARY AND CONCLUSIONS

A study has been made of the inactivation of histamine by organs perfused by means of the Starling heart-lung preparation. The degree of inactivation was determined by the effect on the blood pressure of a test animal.

The heart and lungs, hind limbs, small intestine, liver, and kidneys were studied from this point of view. The kidneys were most effective of all the organs studied in eliminating histamine, since they inactivated as much as 25 mgm. in fifteen minutes. The liver was less effective than the kidneys but was more effective than the other tissues studied in inactivating this amine.

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INCREASED SUSCEPTIBILITY TO LOCAL INFECTION FOLLOWING BLOCKAGE OF LYMPH DRAINAGE

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Received for publication February 7, 1935

Lymphatic obstruction in human beings becomes a crippling disease for two reasons. These are, first, the gradual development of elephantiasis and, second, repeated attacks of acute lymphangitis with local disability and general prostration. Such attacks are not invariable in elephantiasis but when they occur they accelerate the proliferative processes and are in themselves a serious cause of disability.

In recent papers, Drinker, Field and Homans (1934) and Drinker, Field, Heim and Leigh (1934) have described the production in dogs of edema and elephantiasis due to lymphatic obstruction, and have presented detailed examinations of the edema fluid as elephantiasis progresses. In those papers, acute inflammatory attacks were mentioned. These occurred spontaneously in the affected legs of certain of the animals, and could be induced by injection of a hemolytic streptococcus into the edematous part, the organism having been isolated from one of the animals early in a spontaneous attack. It is the purpose of the present paper to describe such attacks in detail since they appear to be one expression of a loss of function ordinarily provided by the lymphatics.

Since filariasis is much the most frequent cause of elephantiasis, it is appropriate to review briefly current conceptions of the etiology of the condition and thus to gain an idea of the fundamental identity of the disease in human beings and the condition which develops in dogs deprived of lymph drainage. Lymphatic obstruction in filariasis depends, first of all, on the presence of female filaria in the lymphatics of the affected part. Through some means, not certainly understood, these organisms induce fibrotic changes leading to obstruction. This is added to by dead ova and dead microfilaria. Lymph stasis results and very frequently attacks of lymphangitis. The part enlarges due in the beginning to edema but very soon to fibrous overgrowth. At the present time there is a controversy as to whether elephantiasis due to filariasis can occur as a result of lymph stasis alone or whether bacterial infection is also essential. O'Connor

(1932) has provided an excellent review of the mixture of knowledge and opinion which has caused this controversy. Our observations must not be thought of as settling it. All they show is that dogs which are normally highly resistant to streptococcic infection become locally very susceptible to these organisms if they are injected into a part edematous from lymphatic obstruction, and that just as in human elephantiasis these dogs, in certain instances, experience spontaneous attacks of lymphangitis. Early in these attacks a hemolytic streptococcus may be cultivated from the edema fluid. The bearing of these findings upon the problems of filariasis is for others to settle. It is, however, possible that our experience may clarify the methods to be used in arriving at more satisfactory clinical results.

The attacks of infection. During the past two and a half years we have produced some measure of lymphedema and elephantiasis in a number of animals. Most of these have been sacrificed relatively early in the disease caused by lymphatic obstruction, in order to obtain information available through a terminal experiment with autopsy. Five dogs have been given marked lymphedema and have been studied over long periods. Of these, 4 have developed elephantiasis in greater or less degree, the degree of overgrowth being determined by the appearance of fistulae. Dogs subjected to lymph blockage experience a tremendous dilatation of lymph capillaries, particularly in the skin. These vessels are prone to rupture through the thinned epithelium, and fistulae result which drain and close and then break out again so that the part is never subjected to true stagnation of edema fluid. When fistulae occur frequently, not only does one observe less elephantiatic overgrowth, but local infection, such as we shall describe, is relatively uncommon.

An abridgment of the history of dog 1[1] will provide a characteristic account of our experiences with streptococcic infection in experimental lymphedema and elephantiasis. On May 5, 1933, when permanent swelling of the left hind leg had been established and lymphatics could no longer be found, it was noticed that this dog was unwell. He favored his left hind leg in walking and it was slightly tender. At the time, we failed to recognize what was taking place and within 36 hours the animal was active once more. Ten days later, May 15, the same thing happened again. The attack began at 4:30 p.m. The left leg was swollen, hot, and painful on motion though not so to touch. Three hours later the temperature[2] was 105.8°F. and the leucocyte count in the blood 25,200 per cubic millimeter. Edema fluid flowed freely on puncture. It had a protein

[1] The course of elephantoid growth and the composition of the edema fluid in this animal have been described. He is dog 1 of the first two papers in the bibliography of this paper. Dog 2 is dog 2 in the same papers.

[2] All temperatures are rectal.

content of 2.36 per cent, and 58,300 white cells per cubic millimeter of which 90 per cent were polymorphonuclear leucocytes. Next day fever continued, but on the third day was gone and the animal was entirely recovered. Cultures of the edema fluid and blood made upon the second day were sterile.

The third experience occurred upon June 23, slightly over a month later. At this time the dog was living in the country, as one of several house dogs. He was active and except for the appearance of his hind legs, wholly normal. At 4:00 p.m. on the 23rd it was noticed he was limping. An hour later a hard chill began and the temperature was 103.4°F. It rose steadily and at 8:00 p.m. was 106.7°F. At that time leucocyte counts in blood and edema fluid were 15,800 and 37,800 per cubic millimeter, respectively. A hemolytic streptococcus was isolated from the first specimen of edema fluid at 8:00 p.m., but not again during this attack. Blood cultures were consistently sterile. The animal had fever throughout June 24, but by the afternoon of the 25th was again well.

A fourth attack occurred on July 26, again without the slightest warning. On this occasion a hemolytic streptococcus was again found in pure culture in the edema fluid taken in the first 3 hours of the attack, and blood cultures obtained simultaneously were sterile. On the 28th the dog was entirely recovered.

From May 5, 1933 until November 19, 1934, dog 1 has had 14 of these spontaneous attacks in the left hind leg and 3 in the right leg, which is somewhat edematous and elephantiatic but which has never been pushed as far as the left. Figure 1 gives the detailed findings in a typical seizure, that of September 5, 1933. When this attack occurred the dog was living in the country. At 5:30 p.m. it was noticed he was limping. Fifteen minutes later he had a severe chill. His temperature at 5:55, as shown in figure 1, was 102.2°F. It rose rapidly and by the time the laboratory was reached was 106.4°F. From this point it fell until a little after 2:00 a.m. when a second rise began which subsided during the afternoon and following night. The edema fluid gave positive cultures of hemolytic streptococci during the first 12 hours of observation. It then became sterile and remained so until a final specimen was taken on the morning of September 7, which turned out to be positive. This and one other occasion are the only times upon which a positive culture has been obtained except early in an attack. Blood cultures were taken with each specimen of edema fluid. All were negative.

The record displayed in figure 1 becomes rather more graphic when compared with figure 2. This chart shows the result of injecting 13 cc. of a dilute culture of hemolytic streptococci isolated from the same dog in a previous spontaneous attack. The animal, dog 1, weighed 41.7 kgm. and the left leg into which the organisms were injected was very large and filled

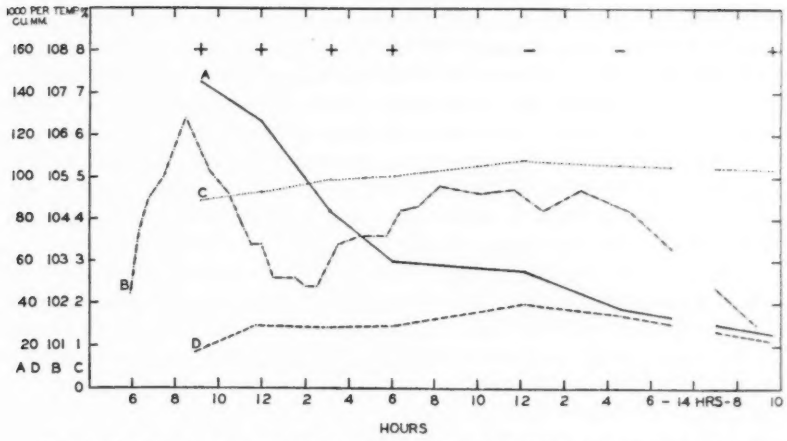

Fig. 1. Details of a typical infectious attack in the left hind leg of dog 1, September 5, 1933. *Line A*, leucocytes in edema fluid in thousands per cubic millimeter; *line B*, rectal temperature in degrees Fahrenheit; *line C*, total protein in edema fluid in grams per cent; *line D*, leucocyte count in blood per cubic millimeter. *Ordinates*, as given for the different curves; *abscissae*, in hours. *Plus signs* indicate cultures of edema fluid positive for hemolytic streptococci; *minus signs*, negative cultures.

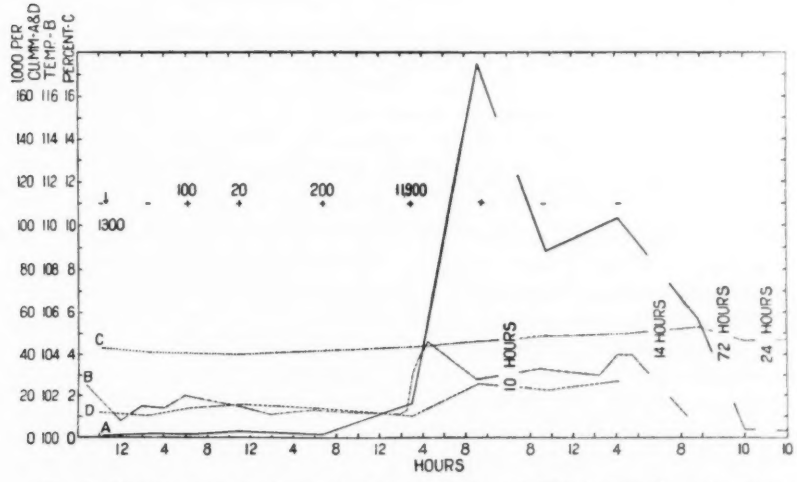

Fig. 2. Details of an infectious attack in dog 1, April 4, 1934, induced by subcutaneous injection of a dilute culture of hemolytic streptococci isolated from this animal in a spontaneous attack. Charting identical with figure 1. At the arrow, 0.3 cc. per kilogram of a dilute culture containing 1300 colonies of streptococci per cubic centimeter was injected in divided amounts under the skin of the left leg. Subsequent figures indicate the number of colonies per cubic centimeter recovered in specimens of edema fluid.

with edema fluid. Control experiments on normal dogs had shown that cultures of this streptococcus containing more than 100,000,000 colonies per cubic centimeter in doses of 0.3 cc. per kilogram were required in order to produce a measurable increase in temperature and blood leucocyte count in normal dogs. In this experiment, 1300 colonies per cubic centimeter were used. We have, however, seen reactions after injection of this streptococcus in concentrations as low as 25 colonies per cubic centimeter, the culture being injected subcutaneously in a lymphedematous leg in an identical amount per kilogram.

Following the injection shown in figure 2, there were no symptoms until 30 hours had passed. The dog then had a chill and a typical attack. There was a tremendous outflow of leucocytes into the edema fluid (curve *A*, fig. 2) and at the height of this reaction no organisms were recovered by

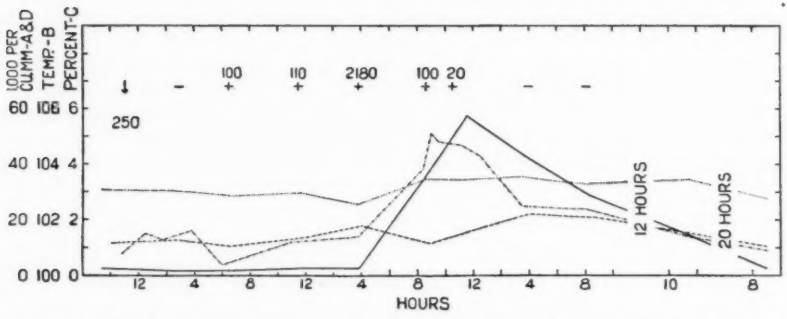

Fig. 3. Details of an infectious attack in dog 2, December 15, 1933, induced by subcutaneous injection of 0.3 cc. per kilogram of a dilute culture containing 250 colonies per cubic centimeter of a hemolytic streptococcus isolated from dog 1 during a spontaneous attack. Charting identical with figures 1 and 2.

direct plating but a few were found later by plating from bouillon tubes. Subsequent cultures were negative. As in spontaneous attacks blood cultures were taken regularly and were sterile.

The experiment shows growth of the organism planted in the edematous leg and a characteristically explosive reaction when, however, the number of colonies in the edema fluid was not great.

Figure 3 is the graphic record of another similar experiment. In this case the same organism was injected into the edematous and elephantiatic leg of dog 2. Dosage was the same per kilogram and the number of organisms in the culture used equalled 250 colonies per cubic centimeter. The result is very similar to that seen in figure 2. The organisms increased in the edema fluid for some hours and when a very moderate number was reached, 2180 colonies per cubic centimeter, a pronounced reaction occurred with a heavy outflow of leucocytes in the edema fluid and rather prompt sterility.

The attacks, both spontaneous and induced, have had certain characteristics in common. First of all, blood cultures have never been positive in the transient seizures. Second, the attacks with a single exception have been over in 3 days. Third, a hemolytic streptococcus was the single organism isolated repeatedly from the edema fluid during attacks. Staphylococci and diphtheroid organisms have been obtained occasionally but have had no uniform place in the history of the disease and have been considered to be contaminating organisms. Fourth, except upon two occasions, both in the case of dog 1, no streptococci have been isolated from the edema fluid later than the twelfth hour after the onset of an attack. Fifth, repeated attempts to find hemolytic streptococci in the edema fluid between attacks have failed completely.

The fourth of these "common characteristics" which mentions two exceptions requires comment. It has been shown in figure 1 that in a spontaneous attack experienced by dog 1, a positive culture of hemolytic streptococci was secured 40 hours after the onset of the attack and following two collections of fluid which proved sterile. No reason can be offered for this other than the entrance of the needle into a small pocket of fluid which still contained living organisms. Until very recently the attacks of lymphangitis, though severe, have been brief. This is the ordinary experience in human cases. But on November 23, 1934 a more serious seizure was encountered. Four days previously, dog 1 wandered off with another dog belonging to one of the authors. In the next days he was heard from twice and at an increasing distance from home. He was recovered on the morning of the 23rd and had lost greatly in weight and strength. It was at once noticed that the dog was lame in the right hind leg. His temperature was 103.9°F. Edema fluid from the right leg contained 171,000 cells per cubic millimeter and a hemolytic streptococcus was recovered from it. As the left leg was also hot, fluid was taken from it. This fluid contained 78,000 cells per cubic millimeter and was also positive for hemolytic streptococci.

A very serious illness now began in which cultures of edema fluid from both legs were repeatedly positive. Abscess formation occurred in the right leg. This evacuated spontaneously on November 26. The illness became a series of attacks, both legs being involved. Every three or four days a typical seizure occurred with high fever and great prostration. On December 14, under nembutal anesthesia, multiple incisions were made in both legs. The fluid which escaped was relatively clear and had a protein content of 4.5 per cent. Defibrinated blood from the animal killed the infecting streptococcus, and it was thought that a rapid renewal of the edema fluid might result in a medium less favorable for growth. This supposition proved wrong. The illness continued and finally the afebrile periods disappeared. On January 26 the dog was anesthetized

and killed. Blood cultures at this time gave a rich growth of hemolytic streptococci, and the same organism was obtained from the edema fluid and from tissues taken from all parts of the body.

The infecting streptococci. A hemolytic streptococcus was isolated first from dog 1 on June 23, 1933. An organism identical in cultural characteristics was then isolated repeatedly from this dog and from two other animals in similar condition. This streptococcus was killed by the whole blood of normal dogs and of dogs that had had attacks of lymphangitis. It grew in the edema fluid taken between attacks. It was avirulent for mice, and when inoculated subcutaneously in normal dogs produced no reaction until enormous dosage was reached. Inoculated in tissue with blocked lymphatics a characteristic attack was induced.

Many attempts were made to obtain this organism from edema fluid between attacks. All were failures. Cultures from muscle and lymph nodes were likewise negative.

The series of attacks in dog 1 which began on November 23, 1934 were due to a different hemolytic streptococcus. This organism at first produced isolated attacks and finally a fatal illness. It proved very virulent for mice, contrasting markedly with the first streptococcus. Whereas in the case of the first organism the undiluted culture was non-fatal for mice, as few as twenty of the second streptococcus caused death. Injected subcutaneously in normal dogs it caused local disturbance with fever in relatively small dosage. The reaction was, however, mild when compared with the result of injecting such an avirulent organism as streptococcus 1 into lymphedematous parts.

Discussion. The experiences which have been described bring out, first of all, the extreme susceptibility to streptococcic infection which follows lymphatic obstruction. Not only is infection easily possible but, as in the case of the first streptococcus encountered, the infecting organism may be of low virulence and yet capable of causing severe illness. It has not been possible to learn the origin of the infecting streptococci. Large amounts of culture of the first organism have been injected intravenously with no result. Spontaneous attacks on three occasions followed severe muscular work, but efforts to induce an attack by heavy exercise have not been successful.

The uniformity with which streptococci have been isolated from edema fluid is not in accord with human experience. But here again our procedure has been somewhat different. Edema fluid can be collected from many cases of human elephantiasis if the part is allowed to be dependent for a half hour prior to puncture. In order to get fluid for culture it should not be necessary to inject salt solution and withdraw it. Furthermore, it is imperative that cultures be made as early in the attack as possible. The induced attacks shown in figures 2 and 3 indicate forcibly enough that

the highest concentration of organisms occurs with the very onset of the disturbance.

SUMMARY

1. Dogs subjected to lymphatic blockage develop a local susceptibility to infection by hemolytic streptococci.

2. Attacks of chills and fever with local inflammation occur spontaneously in such animals.

3. A hemolytic streptococcus can be isolated from the edema fluid early in such attacks.

4. Blood cultures have never been positive during characteristic brief attacks.

5. The source of the infecting organism has not been determined. Large dosage through the blood stream has not produced attacks.

6. These results indicate the importance of normal lymphatic drainage in resisting infection.

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THE USE OF HYPERTONIC SUCROSE SOLUTION INTRAVENOUSLY TO REDUCE CEREBROSPINAL FLUID PRESSURE WITHOUT A SECONDARY RISE

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Received for publication January 16, 1935

The methods used for reducing high intracranial pressure fall into three groups: 1, direct relief of pressure by opening the skull; 2, cerebrospinal fluid drainage, usually by lumbar puncture; and 3, dehydration of the brain and ventriculo-subarachnoid system by changing, either directly or indirectly, the composition of the blood. Each of these methods is subject to distinct limitations.

Inasmuch as the cranial contents are not fluid and the cranial cavity is subdivided by more or less rigid structures (tentorium and falx cerebri), operative decompression may fail to reduce the pressure equally throughout the brain and lead to local congestion, sudden swelling and edema, which entirely defeat the purpose of the operation (Heuer, 1929; Munro, 1934). Lumbar puncture, especially when the intracranial pressure is high, is regarded by some as a hazardous procedure (Cushing, 1909; Homans, 1932; Bailey, 1932; Masserman and Schaller, 1933); although Masson (1929), in an analysis of 200 cases, tends to minimize its dangers. The third method, introduced by the experiments of Weed and McKibben (1919) and applied clinically with favorable results by Haden (1919), Sachs and Belcher (1920) and others, has been complicated by the toxicity (Cushing and Foley, 1920; Foley and Putnam, 1920) and secondary rise in cerebrospinal fluid pressure caused by the substances used for dehydrating the brain. The experiments which will be described in this paper indicate that the latter effect may be avoided by using hypertonic sucrose intravenously.

A secondary rise of intracranial pressure from oral administration of sodium salts (200 cc. of 19 per cent Ringer's solution) was seen by Ebaugh and Stevenson (1920) in a patient whose intracranial pressure changes were followed by placing an inverted tambour over the area of bone defect remaining from a subtemporal decompression. The secondary rise, now commonly known to follow the administration of hypertonic sodium chloride by mouth or by vein, was a constant sequence. With saturated

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sodium sulphate by mouth, the pressure was effectively reduced throughout the period of observation lasting 9 hours. With 200 cc. of 30 per cent glucose injected intravenously, the drop in pressure was small; on the other hand, no secondary rise was seen after $8\frac{3}{4}$ hours. Experimental and clinical evidence has since shown that glucose also produces a definite secondary rise in pressure (Browder, 1930; Milles and Hurwitz, 1932; Jackson, Kutsunai, Leader and Joseph, 1933; Masserman, 1934), an effect which was missed by earlier investigators primarily because their experiments were not continued over a sufficiently long period after the injections were made. This secondary effect indicates that the giving of hypertonic glucose for the reduction of intracranial pressure may be inadvisable, since the treatment at one stage aggravates the condition which it is intended to relieve. The tendency has therefore been to discard intravenous therapy (Dandy, 1933) and to depend upon methods which do not produce a secondary rise in pressure. Fay (1924, 1925) has recommended the use of magnesium sulphate by mouth or by rectum, together with restricted fluid intake. Although this eliminates the secondary rise in pressure, the method leads to vomiting or purging and, as pointed out by Fay himself, the dehydration which it produces may be dangerous when the patient's condition is complicated by hemorrhage or shock.

Hughes and Laplace (1930) reported that the cerebrospinal fluid pressure in dogs could be reduced for 3 to 4 hours by intravenous injection of 25 per cent sodium arabinate solutions. To our knowledge, sodium arabinate has never been used clinically, probably because its advantages do not warrant the trouble involved in its preparation.

Of all the hypertonic solutions which have been tested as intravenous agents for reducing the cerebrospinal fluid pressure, concentrated glucose has come to be regarded as the most satisfactory (Howe, 1925), although, as pointed out above, it has more recently been shown to produce a secondary rise in pressure. This drawback can probably be attributed to the comparative freedom with which glucose passes from the blood into the spinal fluid. Since changes in the glucose level in the blood are reflected, after a lag, in the spinal fluid (Fremont-Smith, Dailey, Merritt, Carroll and Thomas, 1931), intravenous injection of hypertonic glucose will raise the concentration of sugar in the spinal fluid (Gregersen and Wright, 1935) and increase the osmotic pressure of the latter. Subsequently, when the sugar level in the blood falls, this increase in osmotic pressure presumably causes the accumulation of fluid and the recurrence of high pressure in the ventriculo-subarachnoid system. The degree to which the glucose diffuses into the brain itself has not been determined. Other conditions remaining unchanged, the abnormal pressure is eventually relieved (Masserman, 1934) by the diffusion of glucose out of the spinal fluid and by the utilization of the sugar by surrounding tissues.

It is clear from the above considerations that, for the reduction of cerebrospinal fluid pressure and intracranial tension, a substance is desired which: 1, is non-toxic when given intravenously in high concentrations; 2, produces rapid reduction in the pressure of the cerebrospinal fluid; 3, maintains the period of low pressure for several hours; and finally, 4, does not cause a secondary rise in pressure. To be effective in rapidly drawing fluid from the tissues as indicated by 2, the substance must increase the osmotic pressure of the blood markedly. Conditions 3 and 4 require that it should not leave the blood, or at least that it should not diffuse to a significant degree into the brain or spinal fluid.

From the work of Keith (1924) and Keith and Whelan (1926), and others, as well as from the experience which one of us (M. I. G.) has had with 50 per cent sucrose solutions given intravenously to produce rapid dehydration in dogs, sucrose appears to satisfy the first requirement. This fact, together with the observations of Keith, Wakefield and Power (1932), that in man sucrose is excreted rapidly and completely, indicated that it does not enter the tissues to any great extent, and suggested that it might satisfy requirements 3 and 4.

METHOD. Except for some preliminary experiments on cats, we used dogs exclusively. All animals were deprived of food and water for 15 hours before the experiment.

Ether, dial (Ciba) and sodium amytal (Lilly) were tried as anesthetics. Ether was unsatisfactory for long experiments. Dial caused a troublesome nasopharyngeal exudate, and a gross increase in the cells of the spinal fluid. For example, a dog was given 0.7 cc. dial per kgm.; 7½ hours later the spinal fluid obtained by cisternal puncture contained 1850 white cells per cu. mm. Sodium amytal was used in the majority of experiments. Fifty to 60 mgm. per kgm., dissolved in distilled water to make a 10 per cent solution, were injected intraperitoneally; when increased respiration or slight movements of the legs occurred, small additional doses (0.1 to 0.25 gm.) were given. This anesthetic did not produce any large increase in the number of cells in the spinal fluid. Cisternal puncture in a dog which had been under sodium amytal for 12 hours disclosed a crystal-clear cerebrospinal fluid at 135 mm. pressure, with 20 white cells and 110 red cells per cu. mm. In all our experiments except one, the repeated doses of amytal given to prevent shivering or bodily movements had no apparent effect upon spinal fluid pressure. In two dogs, however, shivering could not be prevented without greatly depressing the respiration.

Prolonged measurements of the cerebrospinal fluid pressure require strict asepsis in order to avoid the pressure changes attending meningitis (Hughes and Laplace, 1930). The skin over the back of the neck was incised and draped with sterile towels; arterial (femoral), venous (femoral) and tracheal cannulae were introduced under aseptic precautions. (The

tracheal cannula was necessary since the mouth was held closed in such a way that the tongue at times interfered with respiration.) After the sterile operation, the dog's head was fixed firmly in a Brodie head holder.

Sucrose (Merck), 50 per cent solution in distilled water, boiled for 5 minutes in a sterile flask, was injected through the femoral venous cannula from a sterile buret, usually at the rate of 5 cc. per minute. In a few experiments this rate of injection was varied in order to determine its effect upon the spinal fluid pressure. Before being injected the solution was warmed to approximately body temperature.

For measuring spinal fluid pressures, we employed a simple 1 mm. bore U-shaped glass tube filled with Ringer's solution. With this manometer, a 100 mm. pressure change involved the displacement of less than 0.08 cc. of fluid.

An 18-gauge needle was used for cisternal puncture. It was fitted on an ordinary T-shaped, three-way metal stopcock which led below through a short piece of rubber tubing to the short arm of the manometer. The outlet of the stopcock opposite the needle was closed with a brass plug, drilled for a watertight fit with a wire obturator, which was bevelled at the end and of such length that when thrust in as far as possible its tip was flush with the tip of the needle. After the cisterna magna was punctured, the obturator was withdrawn to a point which just permitted the stopcock to be turned, connecting the needle with the manometer. This method prevents the loss of any spinal fluid before the pressure is read. Capillary attraction in the manometer was found to be 2 cm.; the manometer scale was therefore set so that its zero mark was 2 cm. above the needle. A fall of 3 cm. in the manometer fluid was sufficient to fill the needle after withdrawal of the obturator. Allowance was made for this volume of fluid displaced by the obturator by filling the manometer to a point 3 cm. above the estimated cerebrospinal fluid pressure (110 to 120 mm. in most dogs) before the puncture was made. *All parts of the pressure-recording system, as well as the Ringer's solution used in the manometer, were sterilized carefully before each experiment.*

The pressure was read at least every two minutes. The readings were always taken at the height of the expiratory rise in pressure. Free respiratory and pulse variations were accepted as indications of an open connection with the cisterna. The cerebrospinal fluid pressure was observed for an hour or more before sucrose was injected, and for 10 to 13 hours subsequently. At the conclusion of the experiment, spinal fluid was removed and red and white cell counts made.

A catheter was passed into the bladder at the beginning of the experiment, and urine measured every 15 minutes. Rectal temperature, heart rate and respiration were noted every 15 minutes. Body temperature was controlled with a heating pad, or with a fan and wet towels on hot days.

Continuous blood-pressure records were obtained from the femoral artery with the use of a large cannula, 5 per cent sodium citrate solution, a mercury manometer and a slowly moving kymograph. In 3 experiments a Hürthle membrane manometer was tried; and in a few experiments the taking of the blood pressure (see figs. 2, 5 and 6) was omitted altogether in order to be sure that loss of small quantities of blood or injection of small quantities of citrate from the wash-out system were not affecting the results, and also because the manipulation necessary for washing out the arterial cannula sometimes disturbed the animal enough to cause changes in cerebrospinal fluid pressure.

EXPERIMENTS AND RESULTS. Twelve experiments were done on cats and 31 on dogs. In addition, 3 dogs were discarded at the beginning of the experiment because of the appearance of blood in the cerebrospinal fluid.

Control experiments. Four control experiments were carried out on dogs under sodium amytal. These experiments all showed a gradual rise in the cerebrospinal fluid pressure of 30 to 45 mm. Ringer's solution during the 10 to 11 hours of observation. This is in agreement with the experiments of Hughes and Laplace (1930), who found that with sodium amytal anesthesia "when any change in the original pressure occurred, it was in the positive direction." Figure 1 shows the most irregular of these controls and is selected to illustrate some of the causes likely to produce variations in spinal fluid pressure. It may be seen that there are several sharp peaks of short duration associated with the manipulation of the animal (intraperitoneal injections, washing out arterial cannula, etc.). These variations in pressure were to some extent eliminated in later experiments by more careful handling, particularly when the anesthesia was light and additional amytal was being injected intraperitoneally. We found no indication that the pressure varied with the depth of anesthesia, unless the anesthesia was so light as to allow shivering and bodily movements or so deep as to affect the respiration to a marked degree (see p. 91).

Spinal fluid removed at the beginning of this experiment was clear, but contained 200 red cells per cu. mm. In most of the experiments 200 to 300 red cells per cu. mm. were present in the fluid removed at the time of puncture. These may have entered the needle as it passed through the muscles, or they may have come from the cisterna magna. Whenever a vessel of any size was injured there was an immediate sharp rise in pressure and bloody spinal fluid soon appeared in the rubber tubing and the manometer.

In the experiment illustrated in figure 1, six hours after the cisterna magna was punctured, there were 150 white cells per cu. mm., and no red cells in the spinal fluid; at the end it contained 450 white cells per cu. mm. In contrast to these results, it may be well to mention that in our early cat experiments, where no aseptic precautions were taken, we frequently found

several thousand white cells per cu. mm. in the spinal fluid. An experiment described above (p. 84) indicates that the increase in white cells in the fluid was not due to amytal alone. The slow steady rise of pressure seen in figure 1 may have been associated with irritation of the meninges, due to the presence of the needle (Grant, 1929). We do not believe it can be ascribed to infection, in view of the careful aseptic technique employed. *The gradual rise in pressure observed in control experiments should be kept in mind in evaluating the changes in pressure under the conditions of the following experiments.*

Sucrose experiments. Results obtained from 160 cc. 50 per cent sucrose given intravenously in 30 minutes to a dog weighing 13.3 kgm. (6 gm. sucrose

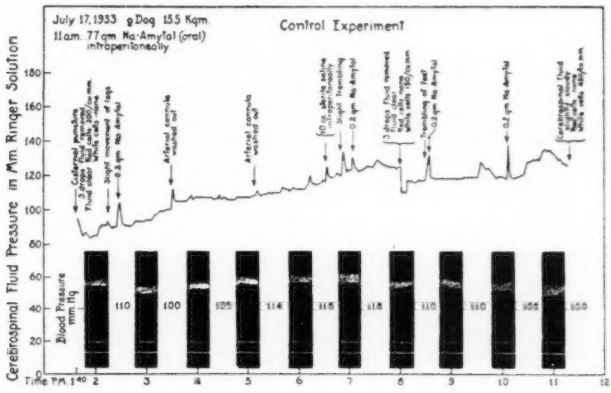
July 17, 1933 g Dog 13.5 Kgm.
11 a.m. .77 gm. Na-Amytal (oral)
intraperitoneally
Control Experiment
Cerebrospinal Fluid Pressure in Mm Ringer Solution
Blood Pressure mm Hg
Time P.M. 1 2 3 4 5 6 7 8 9 10 11 12

Fig. 1. Showing the gradual rise of cerebrospinal fluid pressure which occurred in all control experiments under sodium amytal anesthesia.

per kgm.) are set forth in figure 2. Control readings obtained for 67 minutes prior to injection showed an average cerebrospinal fluid pressure of 78 mm., with a maximum variation of less than 10 mm. during the whole period. Injection of sucrose was attended by a rise of 15 mm., but 20 minutes after the injection was completed the spinal fluid pressure had fallen to less than 30 mm., i.e., 50 mm. below the original normal level. The pressure remained low for 4 hours and 40 minutes, and although it returned for an hour to the original level, it did not reach the height that a control experiment would probably have displayed by this time. Except for the period of light anesthesia from 9:10 to 9:30 p.m., all pressure readings until the end of the experiment, 12 hours and 15 minutes after the injection was over, were below those observed before sucrose was given. There was no indication whatever of the beginning of a secondary rise in pressure.

Unless the sucrose was administered rapidly a rise in cerebrospinal fluid pressure with injection was the exception rather than the rule. Even so, a rise was not invariably produced by rapid injection. In one experiment 26 cc. in 2 minutes led to a momentary increase of 25 mm.; but in that shown in figure 3, 40 cc. in one minute and a half was followed immediately by a sharp drop of 60 mm. When the rate was adjusted at 5 to 6 cc. per minute from the start of the injection, the pressure customarily remained practically unchanged for 10 to 12 minutes and then began a slow fall which changed to a rapid drop as soon as the injection was stopped. On several occasions it was found that the pressure could be kept constant during the injection by increasing the rate at which sucrose was given, indicating that a balance was being maintained between the effect of

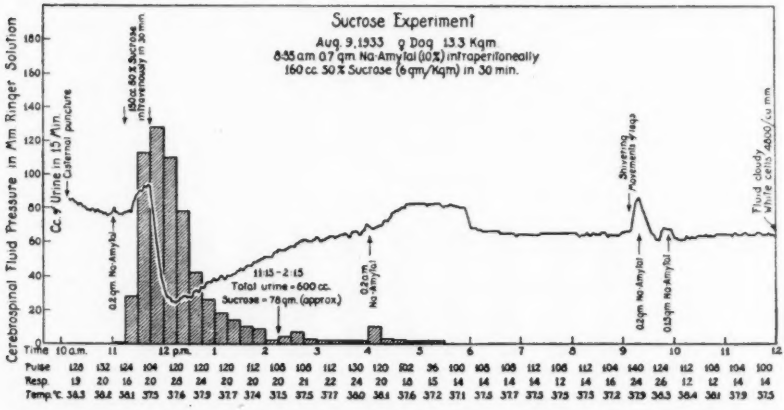

Fig. 2. Reduction of cerebrospinal fluid pressure resulting from the intravenous injection of 6 grams sucrose per kilogram body weight.

increased osmotic pressure of the blood and the effect of increased venous pressure. On no occasion did we observe sudden toxic effects such as those described for sodium chloride by Weed and McKibben (1919) and others. When given slowly sucrose produced no marked change in the heart rate or blood pressure, although there may be a moderate rise in the blood pressure during the period of hydremia which follows the injection (see fig. 3).

From figure 2, it may be seen that 600 cc. of urine were eliminated in 3 hours after the beginning of the sucrose injection, whereas only 160 cc. of fluid were injected. The animal, therefore, lost no less than 440 cc. of body fluid, equivalent to 3.3 per cent of the body weight. Urine passed during active diuresis contained about 78 grams of sucrose, calculated from the difference in reducing material (Benedict's quantitative method) before

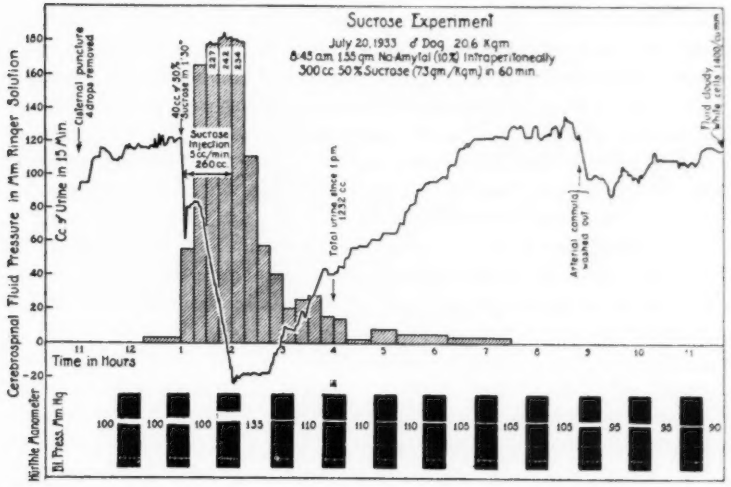
Sucrose Experiment
July 20, 1933 ♂ Dog 20.6 Kgm.
8.45 a.m. 1.55 gm. Na-Amytal (10%) intraperitoneally
300 cc. 50% Sucrose (7.3 gm./Kgm.) in 60 min.
Cerebrospinal Fluid Pressure in Mm Ringer Solution
Cc. of Urine in 15 Min.
Cisternal puncture
4 drops removed
40 cc. of 30% Sucrose in 7 30"
Sucrose Injection 5 cc./min. 260 cc.
Total urine since 1 p.m. 1232 cc.
Arterial cannula washed out
Fluid cloudy
Time in Hours
Nitrite Manometer
Bl. Press. Mm. Hg
100 100 100 135 110 110 110 105 105 105 95 95 90

Fig. 3. Injection of 7.3 grams sucrose per kilogram body weight. The spinal fluid pressure dropped to −20.

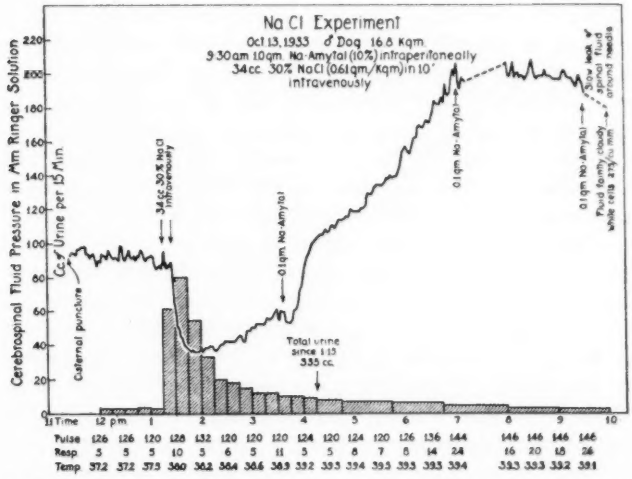
Na Cl Experiment
Oct 13, 1933 ♂ Dog 16.6 Kgm.
9.30 a.m. 1.0 gm. Na-Amytal (10%) intraperitoneally
34 cc. 30% NaCl (0.61 gm./Kgm.) in 10' intravenously
Cerebrospinal Fluid Pressure in Mm Ringer Solution
Cc. of Urine per 15 Min.
Cisternal puncture
34 cc. 30% NaCl intravenously
0.1 gm. Na-Amytal
0.1 gm. Na-Amytal
Slow leak of spinal fluid around needle
Fluid faintly cloudy, white cells 2.5/c.mm.
Total urine since 1.15 335 cc.
Time 12 pm 1 2 3 4 5 6 7 8 9 10
Pulse 126 126 120 128 132 120 120 120 124 120 124 120 126 136 144 146 146 146 146
Resp 5 5 5 10 5 6 5 11 5 5 8 7 8 14 24 16 20 15 26
Temp 37.2 37.2 37.3 36.0 36.2 36.4 36.6 36.3 39.2 39.5 39.4 39.3 39.5 39.3 39.4 39.3 39.3 39.2 39.1

Fig. 4. The temporary fall and subsequent large increase of spinal fluid pressure after the intravenous injection of 0.61 gram sodium chloride per kilogram body weight.

and after hydrolysis. The agreement here between the amount of sucrose injected (80 grams) and the amount excreted is deceiving, for the collections and analyses of urine samples were not accurate enough to give more than

approximate results. Moreover, sterilization of the sucrose solution by boiling may have concentrated it somewhat.[2] Nevertheless, the experiment serves to show that a large proportion of the sucrose is eliminated by the kidney within 3 hours after injection, taking with it almost 4 times as much fluid as was given in the injection. Under these circumstances, it is not surprising that a secondary rise in cerebrospinal fluid pressure fails to appear, for the agents necessary to produce it, namely, sucrose and water, have both been excreted.

Spinal fluid removed at the end of the experiment contained 4800 white cells per cu. mm. It is interesting that the fluid pressure at the end was lower than the original control pressure, in spite of the evidence of meningeal exudation.

Striking results were also obtained with injection of only 3 grams of sucrose per kilogram of body weight. This is illustrated by the following experiment: the normal pressure for a period of one hour before the injection was about 125 mm. Ringer's solution, with a maximum fluctuation of 11 mm. Twenty-six cubic centimeters of sucrose given in 2 minutes raised the spinal fluid pressure momentarily, but in 6 minutes there was a fall to 90 mm. One hundred cubic centimeters were then given at the rate of 6 cc. per minute, and the pressure continued to fall to 42 mm., i.e., 83 mm. below the level at the start. During 12 hours of observation the pressure never returned to the original control level. Seven hours and 20 minutes after the injection the pressure was still 10 mm. below normal; and 11 hours and 45 minutes after injection it was 20 mm. below the original. Fluid removed at this time contained 150 white cells per cu. mm. Blood-pressure records were taken intermittently. As in the experiments of Weed and Hughson (1921), it was evident that the fall in spinal fluid pressure was not associated with a drop in the blood pressure.

Figure 3 illustrates the results obtained with 7.3 grams of sucrose per kgm. (300 cc. 50 per cent). Four drops of spinal fluid removed at the time of cisternal puncture contained 4 white cells and no red cells per cu. mm. The initial pressure was 90 mm., but it soon rose, and during the last hour of the control period remained at about 120 mm. Forty cubic centimeters of sucrose given in 1½ minutes produced no rise in spinal fluid pressure; on the contrary, there was a sharp drop of 60 mm. in 4 minutes. (The blood pressure fell from an average of 100 mm. Hg to 70, but rose again in 5 minutes. Such a transitory drop in blood pressure was seen in only 2

[2] The probability is that more than 80 grams of sucrose were actually injected, for Keith, Power and Peterson (1934) have recently shown that in the dog only 70 to 80 per cent of the sucrose given intravenously can be recovered from the urine; this amount appears within 2 to 3 hours. What happens to the rest of the sucrose is not known. In man (Keith, Wakefield and Power, 1932), sucrose is quantitatively excreted.

experiments where the initial injection was made very rapidly.) The remaining 260 cc. of sucrose were given at 5 cc. per minute as indicated on the graph. At the end of the injection, the spinal fluid pressure was −24 mm., a fall of 144 mm. It rose gradually, reaching the original level 5½ hours after the injection was started. For two hours the pressure was normal or slightly above normal, but then maintained a subnormal level for the rest of the experiment, which lasted another 3 hours.

The animal eliminated 1232 cc. of urine in 3 hours after the injection; 932 cc. more fluid were excreted than injected. The dog therefore lost 4.5 per cent of its total body weight in excreting the injected sucrose.

In only 3 other sucrose experiments did the spinal fluid pressure after injection ever exceed the original level. In one of these, autopsy showed that the needle had been thrust into the medulla. In another, a secondary

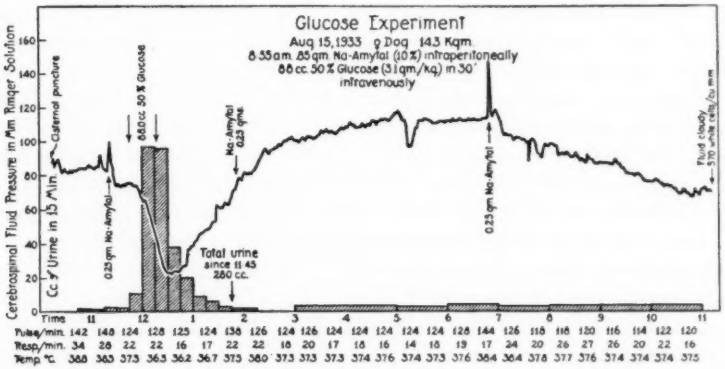
Glucose Experiment
Aug 15, 1933 9 Dog 14.3 Kgm
8.55 am .85 gm. Na-Amytal (10%) intraperitoneally
88 cc. 50% Glucose (3.1 gm/kg) in 30' intravenously
Cerebrospinal Fluid Pressure in Mm Ringer Solution
Cisternal puncture
Cc. of Urine in 15 Min.
0.25 gm. Na-Amytal
88.0 cc. 50% Glucose
Na-Amytal 0.23 gms
Total urine since 11.45 280 cc.
0.23 gm. Na-Amytal
Fluid cloudy — 570 white cells/cu mm
Time
Pulse/min.
Resp/min.
Temp. °C.

Fig. 5. The fall and secondary rise of cerebrospinal fluid pressure induced by the intravenous injection of 3.1 grams glucose per kilogram body weight.

rise of 40 mm. coincided with deep anesthesia and very slow respiration (2 to 3 per minute); a little artificial ventilation promptly caused the pressure to fall. Anoxemia and not sucrose may be held responsible for this increase in pressure (Dixon and Halliburton, 1914). In the third experiment the rise was temporary and so clearly associated with the giving of amytal that one can hardly attribute it to the sucrose. None of our experiments have, therefore, shown that sucrose leads to a secondary rise of pressure which exceeds the original.

The largest dose of sucrose given in any of our experiments was 11.6 grams per kgm. When that amount was injected, the cerebrospinal fluid pressure dropped from 100 to −60 mm., the lowest pressure we ever obtained with sucrose.

Sodium chloride and glucose experiments. Two experiments were done

with sodium chloride in 30 per cent solution for the purpose of comparing its effect with that obtained from sucrose under identical experimental conditions. In one of these (fig. 4) 0.61 gram sodium chloride per kgm. body weight were given. The cerebrospinal fluid pressure was reduced for only 2½ hours, and within 6 hours after the injection it had risen to 200 mm., i.e., 110 mm. above the pressure observed during the control period. This high pressure was maintained for 3½ hours until fluid began to leak out around the needle in the cisterna magna. In the other experiment, in which 1.64 grams per kgm. were injected, the secondary rise in pressure was equally striking, despite the excretion of 660 cc. of urine in 3 hours. At the conclusion of the experiments, the spinal fluids contained respectively 875 and 500 white cells per cu. mm.

The results obtained on a dog given 3.1 grams of glucose per kgm. in 50 per cent solution are presented in figure 5. After a drop in cerebrospinal fluid pressure lasting 2 hours, there was a rise of 30 to 40 mm. above the control level. Secondary rises were similarly obtained in two other glucose experiments.

Discussion. *Toxicity.* In consideration of this question it must be borne in mind that any hypertonic solution, no matter how inherently nontoxic the substance itself, may have harmful effects if given in sufficiently large quantities or very rapidly. Münzer (1898), for instance, in his study of the toxicity of salts, included for comparison some experiments with dextrose, and demonstrated that this harmless sugar as well as the salts caused death in his rabbits when given in sufficiently large amounts. It is clear, therefore, that although a substance may not have outright toxic effects there is a limit to which the body can tolerate the osmotic disturbances produced by it. The doses of sucrose which have been employed in the above experiments on dogs (3 to 7 gm. per kgm. body weight, except one experiment with 11.6 grams per kgm.), and which effectively reduced the cerebrospinal fluid pressure, were only ⅙ to ½ as large as the doses which Keith (see p. 84) and one of us (M. I. G., unpublished results) have repeatedly used on dogs and found harmless. As stated above, the solution was usually given at the rate of 5 cc. per minute, although in one experiment (fig. 3) 40 cc. in 1½ minutes had no visible effect except a sudden fall in the spinal fluid pressure. Keith (1933, private communication) has informed us that he has injected 30 per cent sucrose into human subjects in 25 to 30 cases without observing any harmful effects. One of us (L. T. B.) received 3 grams per kgm. body weight (432 cc. 50 per cent solution) intravenously in 2 hours. During the injection this subject became aware of dryness of the mouth and thirst, which could be relieved by sucking a lemon. Later, he noted also a sense of fatigue, malaise and aching of the calf muscles if he exercised; all these symptoms were identical with those experienced by the same subject when dehydrated in other ways (3 days without water,

20 grams of sodium chloride by stomach tube), and disappeared completely upon taking fluids. It seems, therefore, that the only symptoms which were produced by sucrose are directly referable to the withdrawal of water from the body rather than to any specific toxic effect of the sugar. It is not known how rapidly sucrose may safely be given intravenously in man.

Mechanism. The effect which hypertonic sucrose, glucose or sodium chloride given intravenously and magnesium sulphate given by mouth have upon cerebrospinal fluid pressure is of course only one part of their physiological action. Attention is drawn to certain features of the general disturbance which indicates how the pressure changes are produced.

The immediate result of introducing any hypertonic solution intravenously is an increase in the fluid volume of the blood greater than the volume of the solution injected (von Brasol, 1884; Klikowicz, 1886; Lazarus-Barlow, 1895; Leathes, 1895; Kinsman, Spurling and Jelsma, 1928; Keith, 1924; H. P. Smith, 1925; and others). The hydremia is due to withdrawal of water from the tissues (Tashiro, 1926; Baer, 1926; Skelton, 1927; Ernst, 1930) into the blood, a response to the increased osmotic pressure of the latter. The volume of fluid taken up by the blood is said to be directly proportional to the increase in molar concentration resulting from the injection (Kinsman et al., 1928), but it is apparent that the rate at which the injected substance diffuses into the tissues must be taken into consideration since the movement of fluid into the blood will cease as soon as osmotic equilibrium is established. The osmotic effect of such substances as sodium chloride and glucose, which pass through the capillary wall with relative ease, is greatly curtailed by diffusion.

During the period of hydremia following the administration of sodium chloride, diuresis is facilitated by the high level of salt in the blood and by the dilution of the plasma proteins with water drawn from the tissues (Cushny, 1926; Baird and Haldane, 1922); but diuresis fails to remove more than a fraction (35 to 60 per cent) of the sodium chloride (Münzer, 1898; Padtberg, 1910; Baird and Haldane, 1922), the remainder being stored mainly in the skin (Padtberg, 1910) with less water than is required to dilute it to isotonicity. It is interesting to note that Skelton (1927) found less water in the muscles but more in the skin after hypertonic sodium chloride injections. The storage depots for salt take up and hold water at the expense of other tissues. Moreover, after large quantities of salt have been taken by mouth, ingestion of water produces partly diuresis and partly edema of the tissues in which the excess salt is stored (Baird and Haldane, 1922).

These observations regarding the movement of salt in the body provide an explanation for the temporary fall and subsequent rise in cerebrospinal fluid pressure when hypertonic sodium chloride is injected intravenously

or given by mouth. While the blood is taking up water, it is also losing salt into some of the tissues, which subsequently demand water because of the high osmotic pressure bestowed on them by the salt. That the brain shares in the storage of salt under these circumstances is shown by the observations of Foley and Putnam (1920), that the administration of water produces a much more marked and prolonged rise in cerebrospinal fluid pressure if the animal has been given hypertonic saline several hours previously or the day before.

As in the case of other hypertonic solutions given intravenously, 50 per cent sucrose lowers the spinal fluid pressure because it increases the osmotic pressure of the blood; but a combination of circumstances enables it to have a more prolonged effect than either sodium chloride or glucose. It probably remains in the blood for a longer time after injection since its molecules are larger and less diffusible. It is not broken down to any significant extent or utilized (Folin, Trimble and Newman, 1927) as is glucose, nor is it stored in the tissues as is sodium chloride (see above); sucrose is essentially a non-toxic foreign body once it has entered the blood stream. The reduction of spinal fluid volume produced by its osmotic effect is prolonged by a powerful diuretic action which results in rapid elimination from the body of the sucrose, together with the fluid withdrawn from the tissues (see footnote, p. 90).

Magnesium sulphate when given by mouth or rectum draws water into the gut from the blood, which in turn drains fluid from the tissues, including the brain (Fay, 1924). The fall in cerebrospinal fluid pressure caused by magnesium sulphate therefore actually depends upon realizing a large decrease in the fluid volume of the blood. Whereas this may be of little consequence in the presence of a normal blood volume, it imperils the circulation when the blood volume is already low. Sucrose, on the contrary, produces a temporary state of hydremic plethora during the period when the cerebrospinal fluid pressure is falling. Diuresis removes the excess water from the blood stream in from 2 to 3 hours. The final effect upon the plasma volume must of course be determined by the amount of available body water and by the amount of sucrose which is given. Large doses (15 to 18 gm. per kgm.) produce severe dehydration with a 30 to 40 per cent fall in plasma volume (Keith, 1924); but smaller doses of 3 to 6 grams per kgm. produce either no change in plasma volume of normal dogs or at most a 5 to 10 per cent decrease after 5 to 6 hours (Gregersen).

While 50 per cent sucrose injected intravenously reduces the cerebrospinal fluid pressure markedly in normal anesthetized dogs without producing a secondary rise in pressure, its effectiveness for reducing the cerebrospinal fluid pressure in man is not known. Investigation is under way to determine this point.

SUMMARY

The effects of intravenously injected sucrose, glucose and sodium chloride upon the cerebrospinal fluid pressure have been compared under the same experimental conditions.

Spinal fluid pressures were measured aseptically for 10 to 13 hours on dogs anesthetized with sodium amytal. Control experiments all showed a gradually rising pressure during the period of observation (fig. 1). There was also a moderate increase in the number of white cells in the spinal fluid which was not produced by sodium amytal alone.

Intravenous injection of 50 per cent glucose (3.1 gm. per kgm.) and 30 per cent sodium chloride (0.61 gm. per kgm.) reduced the spinal fluid pressure for only 2 to 3 hours; the pressure then rose above the control level, exceeding it by 40 mm. Ringer's solution (glucose) and 110 mm. (sodium chloride) (figs. 4 and 5).

Three to 8 grams sucrose per kilogram body weight, injected intravenously in 50 per cent solution, reduced the spinal fluid pressure for 5 to 8 hours (figs. 2 and 3). The magnitude of the fall of pressure (50 to 150 mm.) was dependent upon the amount of sucrose given and upon the height of the initial pressure. Greater effects were obtained with higher initial pressures. Although observations were made for 12 hours after injection, there was no indication of the beginning of a secondary rise of pressure exceeding the control level, except in 3 experiments. In these, it could be attributed to definite causes other than the sucrose.

Injection of sucrose induced active diuresis lasting about 3 hours, during which the kidneys eliminated about 4 times as much fluid as was given intravenously with the sugar. With doses of 6 grams per kgm. the body fluid lost by the animal represented 4 to 5 per cent of the total body weight.

The mechanisms by which various hypertonic solutions reduce cerebrospinal fluid pressure are compared and discussed. While temporarily increasing the circulating plasma volume, sucrose reduces the spinal fluid pressure without a secondary rise.

CONCLUSION. Hypertonic (50 per cent) sucrose injected intravenously (3 to 6 gm. per kgm.) is effective in reducing the cerebrospinal fluid pressure in normal anesthetized dogs for 5 to 8 hours without causing a secondary rise exceeding the initial pressure.

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THE EFFECT OF INTRAVENOUS INJECTION OF SUCROSE AND GLUCOSE UPON THE REDUCING POWER OF CEREBROSPINAL FLUID, BEFORE AND AFTER HYDROLYSIS

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Received for publication January 16, 1935

The purpose of the following experiments was to determine whether or not intravenously injected sucrose passes into the cerebrospinal fluid. This problem is pertinent to an understanding of why intravenously injected hypertonic sucrose, like hypertonic glucose or sodium chloride, reduces the cerebrospinal fluid pressure, but unlike these two substances, causes no secondary rise exceeding the initial pressure (Bullock, Gregersen and Kinney, 1935). The commonly accepted explanation of the secondary rise has been that the injected substance which produces it diffuses readily into the cerebrospinal fluid and brain; when the concentration in the blood stream falls, the brain remains hypertonic and becomes edematous (Fay, 1924; Masserman, 1934). If this explanation is correct, it seems justifiable to presume that sucrose does not enter the spinal fluid in appreciable amounts. The same conclusion may be urged from a consideration of the excretion of intravenously injected sucrose as contrasted with that of glucose or sodium chloride. The two latter substances are eliminated only in part (50 per cent or less) (Münzer, 1898; Padtberg, 1910), the remainder being stored, at least temporarily, in tissues which may subsequently swell if water is available (Baird and Haldane, 1922). But sucrose appears not to be stored even temporarily (Keith, Wakefield and Power, 1932); the kidneys eliminate most of it during an active diuresis lasting 2 to 3 hours, and consequently, relatively little can be present in the tissues. Furthermore, since the blood-brain and blood-spinal-fluid barriers are notably less permeable than other blood-tissue barriers, only a small fraction of the remaining sucrose would presumably find its way into the brain and spinal fluid. To test this point, the cisternal fluid from dogs, which had been given intravenous injections of sucrose 1 to 3 hours previously, were analyzed for reducing sugar before and after hydrolysis. As will be shown, no positive evidence of sucrose could be found. But when glucose was injected, a marked hyperglycorachia was observed.

METHODS. Twenty-seven experiments were done on 13 dogs which had been kept in the laboratory for periods of from several months to two

years in connection with other investigations. Eight of the animals had double submaxillary fistulae of long standing (1 to 2 years), three were splenectomized, and two had been used for testing the disappearance rates of vital dyes from the blood stream. All dogs were in excellent health and for purposes of the following experiments were regarded as "normal."

Cerebrospinal fluid was obtained by cisternal puncture (sterile) as quickly as possible (2 to 4 minutes) after giving a small dose (0.3 to 0.4 cc. per kgm.) of nembutal intravenously. In every instance the fluid was crystal clear. All samples were promptly centrifuged for 10 minutes at 3000 r.p.m. in order to make sure that cells were not included in the fluid taken for analysis. The fluids were analyzed (triplicate) as soon as possible after collection. With one exception (August 31), a single puncture was made in each experiment. The results were therefore not affected by the increased rate of formation of cerebrospinal fluid, which occurs when the cisterna magna is tapped (Spurling, 1929; Flexner and Winters, 1932; Flexner, 1933a, b).

Intravenous injections were made into the femoral vein with aseptic precautions. The sucrose and glucose solutions, buffered to pH 7.0, were given in 50 per cent concentration.[1] They were warmed to approximately body temperature before injection and given at 10 to 20 cc. per minute, the rate depending roughly upon the size of the animal. The doses ranged from 6 to 12 cc. or 3 to 6 grams per kilogram body weight (see tables 2 and 3).

CHEMICAL METHODS. *Glucose.* Folin's micro-method (1932) for sugar analysis was adopted because it was found to give almost the same results for glucose and invert sugar. The color comparisons were at first made in a Duboscq colorimeter with a yellow light filter, against a 100 mgm. per cent glucose standard diluted in a Greiner tube (Rothberg and Evans, 1923b) to approximately the color strength of the unknown. When known solutions of 25, 50, 100, 200 and 300 mgm. per cent glucose were compared with a 100 mgm. per cent standard in this manner, the results were too high (40 and 65) for the 25 and 50, and too low (170 and 260) for the 200 and 300 mgm. per cent solutions. Perhaps these results may be explained by the fact that Prussian blue is not the only substance present which lends color and density to the solution. Rothberg and Evans (1923a) stressed the importance of using water-clear reagents in order to obtain satisfactory results with their modification of the Folin-Wu method. In the Folin micro-method this condition is not satisfied since the final solution contains, besides Prussian blue, an excess of the yellow potassium ferricyanide and colloidal iron-gum ghatti. When a series of samples ranging from 25 to 300 mgm. glucose are adjusted by dilution to similar shades of

[1] These solutions were kindly supplied by Eli Lilly and Company.

blue, these reagents are obviously present in decreasing concentration as we pass from the 25 to the 300 mgm. per cent glucose solution. It is clear, therefore, that in order to obtain the correct value for any solution, it is necessary always to use a standard which corresponds closely in concentration to the "unknown."

The inconvenience of preparing a series of standards with every analysis was eventually eliminated by measuring the depth of color in terms of optical density with a spectrophotometer. This instrument has been used by Teorell (1931) in a modification of the Fiske-Subbarow method of phosphorus determination; and Urbach (1931–1934) has employed the simpler Pulfrich photometer (Heilmeyer, 1924) in various quantitative chemical methods which depend upon a color reaction. The spectral absorption curve of the Prussian blue solution produced by the Folin micro-method is highest in the region of 670 to 700 mμ (fig. 1). This indicates that in carrying out glucose determinations with a simple colorimeter it would be better to use a red filter with maximum transmission in the region from 650 to 700 mμ than to use the yellow picric acid filters commonly employed (Folin and Malmros, 1929; Folin, 1932).

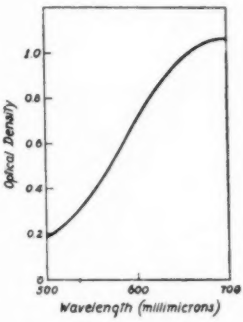

Fig. 1. Spectral absorption curve of the blue-green solution developed by the Folin micro-method for glucose.

Absorption measurements were made at 670 mμ with a König Martens spectrophotometer, using a 1 cm. double quartz cell in which the control cup was filled with water. The samples were all diluted to 50 cc. (within 5 minutes after color development), regardless of color strength, and their optical density determined within 30 minutes. No change in color could be found even one hour after color development.

From the analyses of known glucose solutions ranging in concentration from 25 to 300 mgm. per cent, charts have been constructed (figs. 2 and 3) on which the optical density (at 670 mμ) of the ferri-ferrocyanide solution produced in analysis can be translated into milligrams per cent glucose. The color intensity, measured as optical density, is directly proportional to the concentration of glucose, but the line, instead of passing through the origin, intercepts the ordinate at 0.08. The reagents are responsible for this small absorption in samples containing no reducing sugar.

Sucrose. Inversion of sucrose with hydrochloric acid at 80°C. in a water bath was tried but abandoned. The results were quite inconsistent and always low in comparison with the theoretical values, although the period of inversion was varied from 6 to 24 minutes. There seemed to be destruction of sucrose to non-reducing substances in these dilute solutions.

The method finally adopted for the determination of sucrose is as follows. Two-tenths of a cubic centimeter of the solution to be analyzed is transferred to 10 cc. of water in a 25 cc. volumetric flask; 1 cc. of concentrated hydrochloric acid (36 to 37 per cent) is added and the mixture allowed to

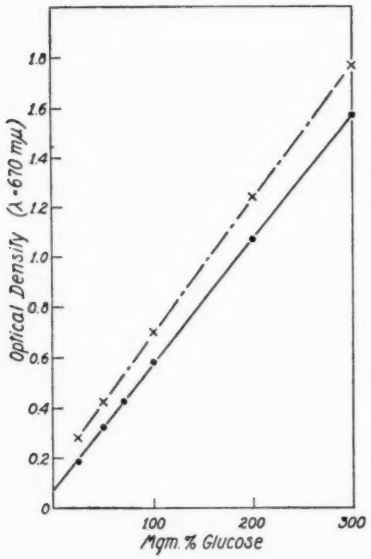

Fig. 2

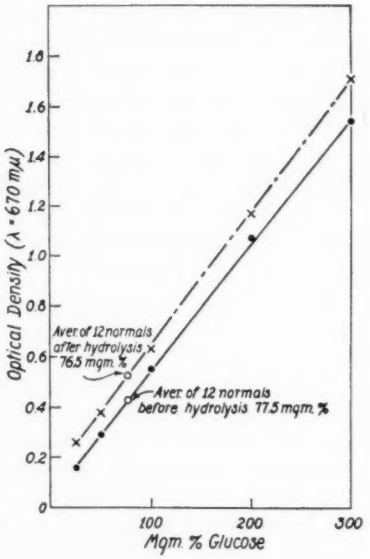

Fig. 3

Fig. 2. Showing the relation between the concentration of glucose and the color developed in analysis by the Folin micro-method. Depth of color expressed as optical density of the solution (1 cm. thick) at wave-length 670 mμ. Plotted from the average results obtained in analyses of 6 series of known solutions (25 to 300 mgm. per cent). •——• = salt-free glucose solutions. ×——× = analyses of known glucose solutions containing sodium sulphate or sodium chloride (0.4 normal).

Fig. 3. Showing the effect of the reagents used in hydrolysis upon the reducing power of known glucose solutions (25 to 300 mgm. per cent). •——• = salt-free glucose solutions. ×——× = the same solutions analyzed after addition of hydrochloric acid previously neutralized to the end-point of phenolphthalein with sodium hydroxide. Showing also that the increase in reducing power of normal cerebrospinal fluid (dog) caused by hydrolysis does not signify an increase in the reducing sugar.

stand overnight at room temperature (Folin, 1925). The acid is then neutralized to the end-point of phenolphthalein with sodium hydroxide and the flask filled to the 25 cc. mark. Five cubic centimeters of this solution are used in analysis for reducing sugar by Folin's micro-method. After color development all samples are diluted to 50 cc. and as soon as

possible the optical density is determined. The averaged results of the analyses of six series of known solutions of sucrose (25 to 300 mgm. per cent) in water are set forth in figure 4.

To demonstrate the validity of this method for estimating sucrose quantitatively in cerebrospinal fluid, known quantities of sucrose were added to different portions of the same specimen of spinal fluid, and the

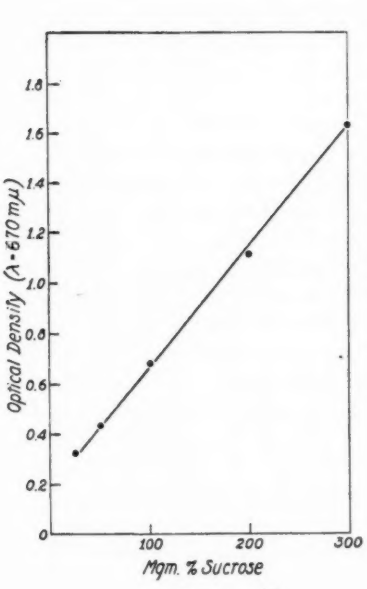

Fig. 4

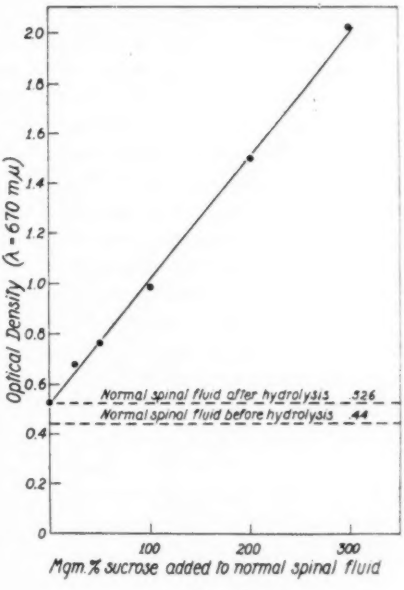

Fig. 5

Fig. 4. Showing the relation between the concentration of sucrose and the depth of color (optical density at 670 mμ) developed in analysis of hydrolyzed sucrose solutions. Average of 6 experiments.

Fig. 5. Determination of sucrose in spinal fluid. Samples of a normal spinal fluid, to which various (known) amounts of sucrose had been added, were subjected to acid hydrolysis at room temperature and analyzed for reducing sugar by the Folin micro-method. The color depth is expressed as optical density at 670 mμ. Average of 6 experiments.

samples analyzed for reducing sugars before and after hydrolysis. The results (fig. 5) show clearly that the reducing power of the spinal fluid is increased by hydrolysis in direct proportion to the amount of sucrose which has been added. A comparison of figures 4 and 5 reveals that the slopes of the lines in these figures are identical.

It was found, however, that even the samples of spinal fluid containing

no sucrose also showed a considerable and consistent increase in the reducing power after hydrolysis, equivalent to about 20 mgm. per cent glucose. From this evidence alone, the hasty and erroneous conclusion might be drawn that normal cerebrospinal fluid contains hydrolyzable reducing material. Further analysis showed that the increase in reducing power is introduced by the reagents used, and that the phenomenon could be duplicated in glucose solutions by merely adding some salt before analysis. If the salt is added after color development has taken place, no change in color intensity occurs. In figure 2 the broken line represents the results of analysis of known glucose solutions to which either sodium chloride or sodium sulphate has been added. In figure 3 the broken line is plotted from results obtained by analysis of three series of known glucose solutions to which the reagents used in hydrolysis (concentrated hydrochloric acid neutralized with sodium hydroxide to the end-point of phenolphthalein) have been added. It is apparent that both procedures lead to a similar increase in the reducing power of the glucose solutions, which again closely approximates the increase obtained by hydrolyzing normal spinal fluid (see below). *The solid line in figure 3, therefore, expresses the relation between the depth of color (optical density at 670 mμ) obtained in analysis and the concentration of reducing sugar (in mgm. per cent glucose), while the somewhat higher broken line gives the same relation after hydrolysis.* The analysis of 12 normal spinal fluids (table 1) before hydrolysis yielded an average optical density of 0.43 and after hydrolysis 0.52. When the latter result is corrected for the effect of reagents, it appears that the glucose value (76.5 mgm. per cent) is nearly the same as that obtained before hydrolysis (77.5 mgm. per cent).

Further support for the opinion that the reagents are responsible for the apparent increase in reducing sugar of normal spinal fluid after hydrolysis was obtained in the following manner: three samples were taken from the same specimen of normal spinal fluid; (a) was analyzed in the usual manner for reducing sugar, (b) was analyzed immediately after the addition of hydrochloric acid previously neutralized with sodium hydroxide to the end-point of phenolphthalein, and (c) was analyzed after treatment overnight with hydrochloric acid. The average results from 6 series of experiments are listed below.

	BEFORE HYDROLYSIS		AFTER HYDROLYSIS
	(a)	(b) neutralized HCl added	(c)
Optical density at 670 mμ.....	0.433	0.519	0.524

Although neutralized hydrochloric acid is ineffective as a hydrolyzing agent, it evidently produces approximately the same change in color

intensity as the addition of hydrochloric acid under conditions permitting hydrolysis.

RESULTS AND DISCUSSION. The results obtained in 27 experiments are summarized in tables 1, 2 and 3. It appears from table 1 that the glucose concentration in the normal spinal fluid of the dog (fasting) shows considerable variation (65 to 93 mgm. per cent). This is consistent with the findings of others (Grayzel and Orent, 1927; Goodwin and Shelley, 1925; Fremont-Smith, Dailey, Merritt, Carroll and Thomas, 1931; Cohn, Levinson and McCarthy, 1933). In considering significant changes in spinal

TABLE 1

Controls. Normal cerebrospinal fluid (dogs)

Reducing sugar in milligrams per cent glucose*

BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
75	79	+4
85	76	−9
90	89	−1
71	69	−2
72	80	+8
70	66	−4
72	63	−9
88	74	−14
93	79	−14
83	81	−2
65	72	+7
69	88	+19
Maximum.....93	89	
Minimum.....65	63	
Average.....77.5	76.5	−1

* The reducing sugar values in tables 1, 2 and 3 have been derived by using figure 3 for converting optical densities obtained in analyses into milligrams per cent glucose.

fluid glucose, this range of normal variations may therefore be a more useful basis for comparison than the actual average figure, 77.5 mgm. per cent.

We have no clear evidence from these experiments of the destruction of reducing substances by hydrolysis such as was observed by Folin and Berglund (1922) in blood, and by Fremont-Smith and Dailey (1925) in spinal fluid. Although the reducing material was less after hydrolysis in more than half the samples (8 out of 12), the average change was only −1 mgm. per cent.

After sucrose had been injected (3 to 6 gm. per kgm.), the reducing sugar in unhydrolyzed spinal fluid was 11 mgm. per cent higher (average than

the normal value taken from table 1. This is probably a real change, since the lowest and highest values obtained, 76 and 104, also showed an increase of the same order. The origin of this rise in spinal fluid glucose

TABLE 2

Sucrose experiments

Fifty per cent sucrose intravenously. Reducing sugar in milligrams per cent glucose.

GRAMS PER KILOGRAM BODY WEIGHT	TIME OF CISTERNAL PUNCTURE AFTER INJECTION		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
	hrs.	*min.*			
3.0	3	15	80	87	+7
3.0	3	10	85	88	+3
3.0	3		88	90	+2
3.0	2	15	104	110	+6
3.0	2		98	101	+3
3.0	2		83	82	−1
3.6	1		88	91	+3
4.5	1		76	89	+13
6.0	1		93	75	−18
Maximum			104	110	
Minimum			76	75	
Average			88	90	+2

TABLE 3

Glucose experiments

Fifty per cent glucose intravenously. Reducing sugar in milligrams per cent glucose.

GRAMS PER KILOGRAM BODY WEIGHT	TIME OF CISTERNAL PUNCTURE AFTER INJECTION		BEFORE HYDROLYSIS	AFTER HYDROLYSIS	DIFFERENCE
	hrs.	*min.*			
6.0		15	107		
3.0		50	162		
3.0	1		175	174	−1
3.5	1		187		
	1	30	165	176	+11
3.0	1	20	112		
	1	20	111		
3.0	2		83		
	2		86		

was not investigated. Hydrolysis increased the amount of reducing sugar in 7 out 9 experiments, but the changes were so small and inconsistent that in our opinion they do not supply positive evidence for the presence of

sucrose. Even if the last experiment were disregarded, the average increase with hydrolysis would be only 4.5 mgm. per cent. From such limited data it is obviously impossible to state definitely whether or not sucrose passes the blood-spinal-fluid barrier.

In the glucose experiments (table 3 and fig. 6) the outcome was in sharp contrast to the results obtained with sucrose. One hour after the injection of 3.5 grams per kilogram (4th expt. in table 3), the spinal fluid glucose was 187 mgm. per cent, i.e., more than twice as great as the extreme upper value found in the normal fluids (table 1). In only one experiment was the concentration of glucose within the normal range. In this case the sample was taken two hours after injection of 3 grams per kilogram. The

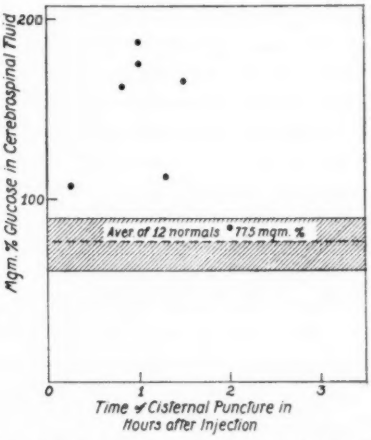

Fig. 6. Solid circles = the sugar concentrations in the cerebrospinal fluid of dogs which have been given hypertonic glucose intravenously. Cross-hatched area = range of glucose concentrations found in 12 normal spinal fluids.

first and last portions were collected separately but the analyses (triplicate) still agreed within 3 mgm. per cent. Specimens from experiments 3 and 4 in table 3 were also tested for reducing sugar after hydrolysis. At the time of the other four experiments the possible interest of the effect of hydrolysis was unfortunately not appreciated. The two results obtained do not indicate any significant change in the amount of hydrolyzable substances present.

It might be suggested that our experiments would be more convincing if control samples of cisternal fluid had been obtained from each animal immediately before the injection of sucrose (or glucose) and compared with samples taken subsequently during the course of the experiments. The objection to such a procedure is that the long period of anesthesia and the

effect of repeated punctures, with removal of fluid, would make the experiments less illustrative of the true changes in spinal fluid occurring in the normal animal. To be sure, the effect of the anesthetic upon the composition of the spinal fluid was not entirely excluded, but it was at least minimized by giving the anesthetic (nembutal) just before (2 to 4 min.) the cisternal punctures were made. Since ventricular pressure influences the rate of cerebrospinal fluid production (Flexner, 1933) and probably also the permeability of the choroid plexus (Spurling, 1929), the results obtained with repeated withdrawal of fluid cannot be regarded as strictly analogous to the effects produced in a closed system which has not been disturbed during the experiment.

From the literature (Flexner, 1934; Cumings and Carmichael, 1934) it is apparent that agreement has not been reached concerning the effect of hyperglycemia upon the concentration of glucose in the cerebrospinal fluid. Munch-Petersen (1930), for instance, claims that hyperglycemia alone does not produce hyperglycorachia. This author found that adrenalin-hyperglycemia, or glucose ingestion combined with adrenalin subcutaneously, was required to raise the spinal fluid glucose 16 to 30 mgm. per cent. On the other hand, a number of investigators, including Goodwin and Shelley (1925), Grayzel and Orent (1927), Fremont-Smith, Dailey, Merritt, Carroll and Thomas (1931), Spurling (1929), and recently Cohn, Levinson and McCarthy (1933), using improved chemical methods, have obtained definite evidence that the spinal fluid glucose concentration rises with hyperglycemia. No doubt some of the conflicting results have been due to differences in chemical methods, the experimental conditions and the animals used. For the purpose of comparing the diffusion of glucose and sucrose into the spinal fluid it is therefore highly significant that our glucose and sucrose experiments were performed under similar conditions and with the same methods throughout.

A question of considerable practical significance is whether or not the blood-spinal-fluid barrier would still display the same degree of impermeability to sucrose after cranial injury (Hoff, 1930). Could hypertonic sucrose be used effectively to break up the vicious circle that is started by such an injury (Cannon, 1901)? In this connection it is important to know whether or not the blood-brain barrier is similar to the blood-spinal-fluid barrier with respect to its impermeability to sucrose.

SUMMARY

In 12 normal spinal fluids obtained from fasting dogs the glucose concentration varied from 65 to 93 mgm. per cent; the average was 77.5. Intravenous injection of 3 to 6 grams of sucrose per kilogram body weight failed to cause a definitely measurable increase in hydrolyzable reducing material in the cerebrospinal fluid (cf. tables 1 and 2). This apparently means that

sucrose does not pass the blood-spinal-fluid barrier. On the other hand, injections of 3 to 3.5 grams of glucose per kilogram produced a marked hyperglycorachia (187 mgm. per cent) within one hour after the injection (table 3 and fig. 6), showing that under similar conditions glucose diffuses readily into the cerebrospinal fluid.

These observations were made with the Folin micro-method for glucose, but spectrophotometric analysis was substituted for colorimetry (Duboscq) in order to avoid the preparation of a series of standards with every determination. The optical density, at 670 mμ, of the blue-green solution produced in the analysis appears to be directly proportional to the sugar content of the sample (figs. 2 and 3).

Evidence obtained indicates that normal cerebrospinal fluid contains no hydrolyzable reducing material. The addition of sodium chloride, sodium sulphate (fig. 1) or neutralized hydrochloric acid (fig. 2) to known glucose solutions increases their reducing power to the same extent as that observed in normal spinal fluid after acid hydrolysis.

CONCLUSION. Sucrose does not appear in definitely measurable amounts in the cerebrospinal fluid of dogs after intravenous injection of 3 to 6 grams per kilogram body weight. Intravenous injection of 3 to 3.5 grams of glucose per kilogram is followed by marked hyperglycorachia.

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THE UPPER LIMIT OF HEARING IN CHIMPANZEE

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Received for publication February 19, 1935

Auditory sensitivity, in its broader meaning, is determined principally by the range of audibility (frequency sensitivity) and auditory acuity (intensity sensitivity). An animal with either of these capacities well developed has a definite advantage in responding to his environment. Naturalists and experimentalists are familiar with the difficulty of approaching a group of primates or other animals without being detected, even when visual and olfactory stimuli presumably are absent. If the cues are auditory and yet beyond the human limits of audibility what is the nature of the stimulus? Is it a matter of auditory acuity or of high frequency sensitivity? Certain animals which have been credited with an extremely keen sense of hearing may be sensitive to vibrations of very low intensity or of very high frequency. There is the further possibility that there may be superiority in both respects. We have insufficient experimental data to know to what extent, if at all, the two capacities are functionally related and coexistent. It is clear, then, that a test of auditory acuity alone does not provide a satisfactory description of auditory sensitivity.

The upper limit of hearing in man has been well established, but with exception of the dog and cat it has not been determined for other mammals. Andreyev (1), using the conditioned salivary reflex method, found the limit for the dog to lie between 36,000 and 38,000 c.p.s., or somewhat lower than results of earlier observations (2). Wever (9) found the limit of audibility for the cat to lie in the region of 10,000 to 20,000 c.p.s. Results of Dworkin, Seymour, and Sutherland (3) indicating "that the cat's range extends beyond that of the dog and far beyond that of man" are qualified by the statement of the experimenters that their loud speaker may not have been functioning properly above 20,000 c.p.s. No reliable data have been obtained for the infrahuman primates other than a few observations by Wendt (8) on monkeys. His tests of high frequency sensitivity, given to only one of five subjects, were incidental to a study of auditory acuity. Responses occurred to tones from a Pierce magnetostriction oscillator[1]

[1] This was the oscillator used with chimpanzees in the present study. Experimental conditions for the monkeys and chimpanzees differed in a few details.

at frequencies from 16,500 ~ to 33,600 ~. Higher frequencies were not available at the time and the upper limit was not determined.

The aim of the present study was to determine the upper limit of hearing in chimpanzees. Three young chimpanzees and three children were used in the same situation.

SUBJECTS. Two of the chimpanzees used in this experiment, Moos and Bimba, no. 11 and no. 26 respectively in the laboratory series, had been used extensively in previous work on absolute intensity thresholds. The third, Alpha (no. 28), had been used very little experimentally and was without experience in auditory work. Her early history has been described by Jacobsen, Jacobsen and Yoshioka in their study of chimpanzee behavior during the first year (5). Information concerning the characteristics and life histories of Moos and Bimba has been presented in a previous report (4). At the beginning of the investigation the ages of these subjects were: Moos, seven years (hypothetical), Bimba, five years (hypothetical), Alpha, three years and five months.

The three children had no known physical defects and had normal auditory acuity for their ages. Subjects L. W. and H. G. were twelve years old, B. E. was five and one-half years. Audiograms for the children and for two of the chimpanzees are presented in figure 1. Those for the children represent results of the usual clinical tests with the 2-A Western Electric audiometer. Examination of the chimpanzees was made in a similar manner except that a food reward was given for correct responses and steps of one decibel attenuation instead of five were used. The latter difference coupled with the fact that both were highly trained probably accounts for the more uniform appearance of the chimpanzee curves.

APPARATUS AND PROCEDURE. In the preliminary training period of the investigation the 2-A audiometer was used as the source of stimuli. High frequency tones were obtained from a Pierce magnetostriction oscillator (6) (7). In this instrument tones result from the longitudinal vibration of nichrome rods, the frequency of vibration being determined by the length of the rod. An outstanding advantage of this apparatus is the constancy of frequency under uniform electrical and temperature conditions. The rods used were cut to approximate a given frequency and then accurately measured in order to determine the actual frequency. Unfortunately, data concerning intensity of the tones are not available. According to Mr. H. W. Lamson, Engineer of the General Radio Company, the intensity level is well above that of the average minimum intensity for human audibility. Frequencies of 14,400 ~ and 16,500 ~ were of sufficient intensity to be disagreeable for most subjects and could be heard without difficulty several hundred feet from the instrument. The following frequencies, expressed in the nearest 100 cycles, were used as stimuli: 14,400, 16,500, 19,000, 20,700, 22,900, 25,000, 30,500, 33,600, and 37,500

cycles per second. The method employed in the present investigation is essentially the same as that used in my previous work on auditory acuity (4). For convenience of the reader the essential features are repeated here.

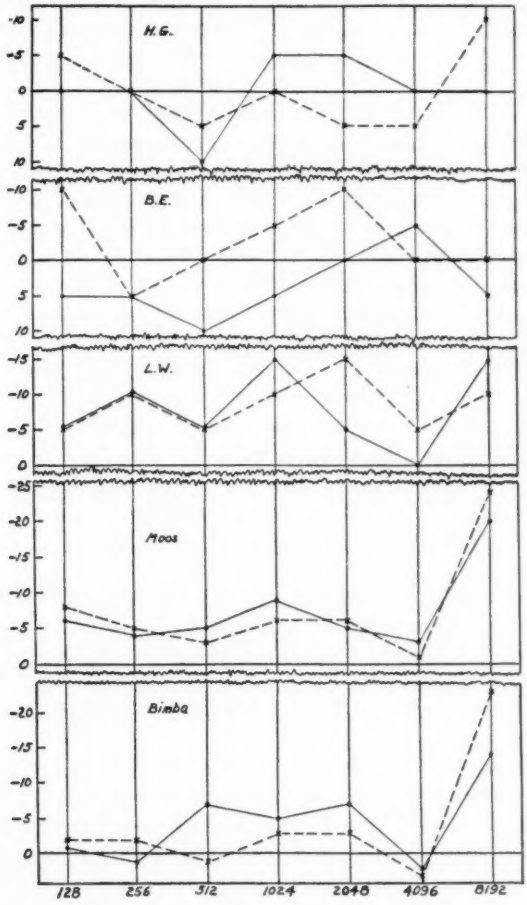

Fig. 1. Audiograms for three human subjects and two chimpanzees. Thresholds for the right ear are indicated by the dots, those for the left by the crosses. The heavy line at 0 represents the average human threshold. Ordinate values are units of intensity above and below the average human threshold, negative values indicating "better" thresholds. Frequencies of complete vibrations are shown along the base line.

Observations were made in a semi-soundproof room with subject and experimenter separated by a one-way vision screen. The subject was

seated at a table facing the screen and directly in front of a food delivery chute and a telegraph key, covered with a hood and sliding panel. The stimulus came from a partially enclosed box 15 to 18 inches from the subject at a point forty-five degrees from the mid-plane in the upper left quadrant. Since it was impossible to keep the subject's head in a fixed position this relationship was not constant although there was little change in distance.

As a preliminary to determination of upper pitch limens each subject was trained to respond to a tone by pressing the key and to refrain from pressing in the absence of a tone. Most of this training was done at a frequency of 8192 c.p.s. with the 2-A audiometer as stimulus source. With Moos and Bimba, because of previous mastery of the procedure, this training was not necessary but as a matter of routine they were required to work through three training periods of twenty trials each with 8192 c.p.s. at a barely audible intensity before passing to the higher frequency tests.

The method of presenting the stimuli after preliminary training was as follows. With the subject seated before the table the experimenter raised the sliding panel, exposing the key, and after three seconds, either presented or did not present a tone. An experimental period consisted of twenty trials, ten of which were without stimuli.

For each correct response the chimpanzees were rewarded with a piece of fruit which dropped into the cup; a false response (response to absence of tone) was unrewarded. For each correct response the human subjects were rewarded with a penny.

Results. None of the subjects used failed to respond to a frequency of 16,500 ~ or less. The frequency at which the formal tests were begun was determined for each subject on the basis of response to several preliminary presentations of successively increasing frequencies. The highest frequency to which a subject uniformly responded was taken as the starting point of the daily test series.

Table 1 shows the total number of trials given to each chimpanzee at various frequencies, the percentage of correct responses, and the number of false responses. Table 2 gives corresponding data for the children. It should be noted that the actual number of stimulus presentations is one-half of the total number of trials and that the percentage of correct responses is based upon stimulus trials only. The non-stimulus trials served as a part of the training against guessing. They are important chiefly as an indication of reliability of the subjects' positive responses. In table 1, for example, Moos was given ten and twenty tone presentations at 25,000 ~ and 27,400 ~, respectively, and an equal number of trials in which the stimulus was absent. He responded to all of the stimuli and made no responses in the non-stimulus trials. At 30,500 ~ he responded in forty-four of the sixty stimulus trials and also in three of the sixty non-stimulus trials. Ordinarily the total number of trials is a multiple of twenty, the

regular experimental series. The three exceptions, which appear in the records of Moos and Alpha, indicate that an experimental period was interrupted by emotional disturbance (temper tantrum) or complete failure and refusal to work at a particular frequency. Alpha, for instance, after almost complete failure at 27,400 ~ refused to work, not only at this frequency but at all others.

TABLE 1

Responses of chimpanzee subjects to frequencies of 22,900~ to 37,500~

FREQUENCY	MOOS			BIMBA			ALPHA		
	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses
22,900				20	100	0			
25,000	20	100	0	20	90	0	20	100	0
27,400	40	100	0	80	70	0	26	23	4
30,500	120	73.3	3	40	30	0			
33,600	111	47.3	2						
37,500	8	0	0						

Percentages of correct responses are based upon the number of stimulus trials only (one-half of the total trials); false responses are responses occurring in non stimulus trials.

TABLE 2

Responses of human subjects to frequencies of 16,500~ to 25,000~

FREQUENCY	L. W.			B. E.			H. G.		
	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses	Number of trials	Per cent correct	Number of false responses
16,500	20	100	0						
19,000	20	90	0				20	100	1
20,700	60	50	5	20	100	0	20	70	0
22,900	20	10	2	40	85	0	60	53	2
25,000				20	0	0	20	0	2

Percentages of correct responses are based upon the number of stimulus trials only (one-half of the total trials); false responses are responses occurring in non-stimulus trials.

Figure 2 is a graphic representation of the performance of all subjects, in which are shown percentages of correct responses at different frequencies and the fifty per cent threshold for each individual. All frequencies lower than those represented on the curves were regularly responded to; there were no indications of the presence of tonal islands or other unusual conditions.

DISCUSSION OF RESULTS. Immediately apparent from inspection of

figure 2 is the fact that the curves for chimpanzees and children form two distinct groups. Two of the animals have records of perfect response at 25,000 ~, a frequency to which none of the children responded. Several human adults, examined briefly in the same situation, never responded to this frequency. In fact, only one of them was able to hear the 22,900 ~ tone; most of them could hear 19,000 ~; while a few of the older subjects failed to hear 14,400 ~. All of the experimental data, as well as the results of these informal tests agree with previous determinations that the upper limit of hearing in man is approximately 20,000 cycles per second. The upper limit for chimpanzees under eight years old, as indicated by tests with three subjects, is near 30,000 cycles per second.

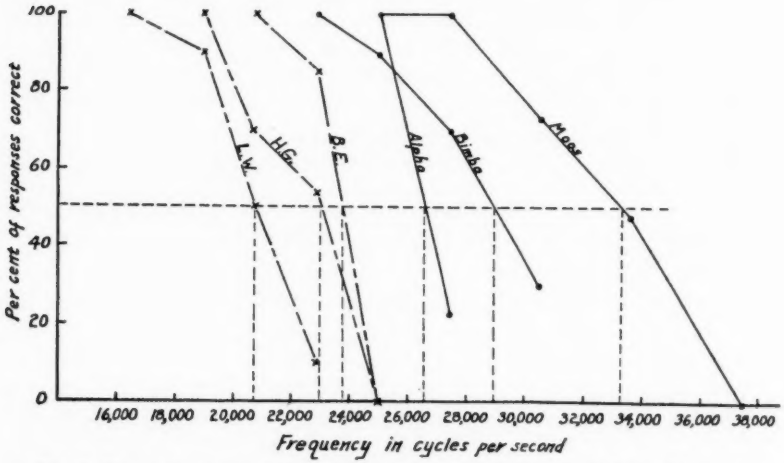

Fig. 2. Upper frequency limens for three human subjects and three chimpanzees. Each limen is indicated by the dotted perpendicular dropped from the intersection of individual curves and the horizontal 50 per cent line.

The factor of practice should be considered in evaluation of the foregoing results. It was stated above that Moos and Bimba were used extensively in auditory acuity experiments while the third subject, Alpha, did not have the advantage of this experience. It is possible that this difference is reflected in the results, probably as the effect of a more serviceable general adaptation as a result of long practice rather than a real change in sensory capacity (no subject had more practice than another with tones above 8,192 ~). Effects of practice, within the present experiment, were negligible in all cases; and the fluctuations from day to day, frequently observed when working with minimum intensities were likewise small.

A comparison of the results presented here with those obtained by Wever for cats reveals some interesting differences. Just how much may be

ascribed to differences in subjects observed and how much to methods employed it is impossible to say. Aside from the fact that the limens for Wever's cats are all lower than those of the chimpanzees, there are large differences in the frequency range over which responses were obtained. The general form of the curves is also different. Whereas the responses of chimpanzees as well as children fell off rather sharply with relatively small increases in frequency, the cats continued to make the "flutter response" at a fairly low level of efficiency over a much broader range.

It is commonly supposed that monkeys, and also chimpanzees, have a keener sense of hearing than man. It is pertinent, therefore, to suggest that this superiority may be determined entirely by sensory capacity for high frequency vibrations. The conclusion needs further confirmation, but we already have the evidence of Wendt (8) that the auditory acuity of monkeys for tones below 8,192 ~ is of the same order as that of man, whereas for tones above 8,192 ~ the monkey excels. The writer (4) has shown that essentially the same relationship holds for chimpanzee and man.

It is believed that valuable results will be obtained from further studies of high frequency sensitivity in primates, especially if such studies can be correlated with morphological and physiological observations.

SUMMARY

Three chimpanzees and three children, trained to respond to a tone by pressing a key and to refrain from response when tone was absent, were tested with nine different tones ranging from 14,400 to 37,500 cycles per second.

The upper limit of hearing (defined as that value of the stimulus to which responses are made in fifty per cent of the presentations) for the chimpanzees ranged from 26,000 ~ to 33,300 ~, and for the children, from 22,600 ~ to 23,700 ~.

The conclusion is offered that difference in auditory sensitivity between man and other primates probably is a result of the latter's relative superiority in reception of high frequency sounds.

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THE RESYNTHESIS OF PHOSPHOCREATINE AFTER MUSCULAR CONTRACTION

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Received for publication February 15, 1935

Evidence has been presented previously (15b) in support of Fiske's (4) theory that the function of phosphocreatine hydrolysis in muscle is that of buffering the lactic acid formed during contraction under anaerobic conditions. It was shown that in the muscles of rabbits which had been on a diet deficient in base, the amount of phosphocreatine hydrolyzed during a tetanic contraction was such that the pH of the muscle fiber would remain constant. On this basis it might be expected that a similar relationship would be found during the recovery process; i.e., the amount of phosphocreatine resynthesized from its hydrolysis products in a given time should be equivalent, in terms of base removed, to the amount of lactic acid concurrently removed. This relation should hold without regard to whether the lactic acid is removed by oxidation, conversion to glycogen, or diffusion into the blood stream.

The technique used previously readily permits the study of the changes taking place in the muscle itself during the recovery process. Experiments were performed on both cats and rabbits. Cats were used because Imrie and Jenkinson (5) have studied the resynthesis of phosphocreatine in this species. The cats were anesthetized with sodium pentobarbital, the rabbits, with sodium pentobarbital or sodium amytal, and ether. The gastrocnemius muscles were dissected free except for their origins, taking care to leave the nervous and vascular connections intact. The peroneal and tibial nerves were cut just distal to their points of emergence beneath the gastrocnemius. It was thus possible to stimulate the gastrocnemius through the nerve without producing contractions of the other muscles of the leg. The portion of the os calcis into which the tendon of the gastrocnemius is inserted was separated from the rest of the bone. By means of a fairly heavy copper wire wrapped tightly around the tendon just at the enlargement made by its insertion into the bone, the muscle was attached to an isometric lever patterned after that described by Martin, Field and Hall (8). The origin of the muscle was held fixed by a steel drill passing through the condyles of the femur. The drill was mounted in a heavy brass base which was clamped to the table top.

In one set of experiments one gastrocnemius was attached to the isometric lever, put under a few hundred grams tension, and tetanized for 30 seconds. Immediately after the stimulation was ended the muscle was frozen. Less than three seconds elapsed between the end of the stimulation and the completion of the freezing. The resting muscle was frozen as soon as possible after the stimulated one, generally within two minutes. In the

TABLE 1

Resynthesis of phosphocreatine and removal of lactic acid from muscles of cats after contraction

Values expressed as milligrams per cent of P and of lactic acid

RESTING MUSCLE			STIMULATED 30 SECONDS NO RECOVERY			DIFFERENCE			TENSION-TIME IN KGM.-SEC. PER GRAM OF MUSCLE
Inorganic P	Inorganic plus Phosphocreatine P	Lactic acid	Inorganic P	Inorganic plus Phosphocreatine P	Lactic acid	Phosphocreatine hydrolysis	Hexosephosphate formation	Lactic acid formation	
21	87	16	37	59	181	16	28	165	12.7
17	94	15	38	63	206	21	31	191	6.7
23	88	11	51	63	180	28	25	169	14.6
22	87	10	38	63	172	16	24	161	18.3
22	90	10	42	63	204	20	27	194	13.1
23	89	13	50	67	194	27	22	181	10.1
Average						21.3	26.2	176.8	12.6
			STIMULATED 30 SECONDS RECOVERY 60 SECONDS						
21	93	10	32	68	165	11	25	155	9.1
23	97	11	30	70	178	7	27	167	12.3
22	99	8	32	74	136	10	25	128	14.9
21	91	13	21	63	133	0	28	120	11.6
22	87	10	20	57	155	−2	30	145	10.7
25	95	10	30	69	152	5	26	142	17.8
Average						5.2	26.8	142.8	12.7

Lactic acid removed..... 34.0 mgm. per cent
Phosphocreatine-P resynthesized..... 16.1 mgm. per cent
Ratio 2.11

other set of experiments the tendon was cut immediately after the end of a similar tetanus of 30 seconds' duration, and the muscle allowed to recover, in the relaxed state, for 60 or 120 seconds before it was frozen. Here also the resting muscle was frozen immediately after the stimulated one. Determinations of phosphocreatine hydrolysis, hexosephosphate formation, and lactic acid formation were made by the methods previously described (15a). The differences in inorganic P and lactic acid content, above the

resting level, measure the amounts of phosphocreatine resynthesized and lactic acid removed.

The data, given in tabular form, show that there is practically no change in the hexosephosphate content during the first two minutes of recovery. However, the removal of lactic acid and the resynthesis of phosphocreatine from its hydrolysis products do take place in large amount. It is also seen that there is a very close correspondence in the rate at which these two processes are taking place. The ratio of lactic acid removed to phosphocreatine-P resynthesized is close to 2.5 to 1.

At pH 5.6, which Rous (14) has found within the mammalian muscle fiber, the formation of one milligram of phosphocreatine-P from inorganic phosphate and creatine removes base equivalent to 2.5 mgm. of lactic acid. Hence if phosphocreatine resynthesis and lactic acid removal are taking place simultaneously in this proportion, the pH of the system would remain constant. Examination of the data readily permits such an interpretation. The quantities of acid and base removed are equivalent; no matter what absolute amount of lactic acid is removed per minute, phosphocreatine is synthesized from its hydrolysis products in equivalent amount.

Imrie and Jenkinson found that phosphocreatine was resynthesized in cat muscle at the rate of about one-half of one milligram per cent per minute, in contrast to the average value of 16 mgm. per cent found here. They cut the nerves supplying the muscles and allowed an hour or more to elapse between the nerve section and the stimulation. It appears, then, that denervation deranges the mechanism for phosphocreatine synthesis in mammalian muscle.

It would be expected, from the work of Eggleton and Evans (3), that the greater part of the lactic acid which disappears from the muscles in the early part of the recovery period diffuses into the blood stream. In this connection it is necessary to compare the resynthesis of phosphocreatine in mammalian muscle with intact circulation, with the anaerobic resynthesis found by Nachmansohn (13) in isolated frog muscle, under conditions in which the muscle cannot lose lactic acid by diffusion. The fibers, however, can, and in all probability do, lose lactic acid by diffusion into the interspaces. Phosphate ion, on the other hand, apparently does not diffuse from the fiber into the interspaces. This is the interpretation that Eggleton (2) puts on the finding that only 20 to 30 per cent of the water of the muscle is available for free diffusion of phosphate. If some 20 to 30 per cent of the muscle substance is composed of interspaces, then this portion of the lactic acid formed during a tetanus could be expected to diffuse out of the fibers afterward. Then this percentage of the phosphocreatine hydrolyzed during the tetanus must be resynthesized afterward in order to maintain the pH constant within the fiber. Nachmansohn (13) and Lundsgaard (6c) both find that about 30 per cent of the phosphocreatine hydro-

TABLE 2

Resynthesis of phosphocreatine and removal of lactic acid from muscles of rabbits after contraction

Values expressed as milligrams per cent of P and of lactic acid

RESTING MUSCLE			STIMULATED 30 SECONDS NO RECOVERY			DIFFERENCE		
Inorganic P	Inorganic plus Phosphocreatine P	Lactic acid	Inorganic P	Inorganic plus Phosphocreatine P	Lactic acid	Phosphocreatine hydrolysis	Hexosephosphate formation	Lactic acid formation
14	96	30	33	60	197	19	26	167
18	95	20	28	68	180	10	27	160
19	102	20	39	75	192	20	27	172
25	101	22	44	80	168	19	21	146
21	90	17	34	60	150	13	30	133
17	85	20	35	56	179	18	29	159
11	99	26	33	61	189	22	28	163
19	90	28	40	69	153	21	21	125
Average						17.7	26.1	153.1
			STIMULATED 30 SECONDS RECOVERY 60 SECONDS					
20	106	23	26	76	158	6	30	135
26	94	33	29	73	167	3	21	134
21	99	16	27	80	117	6	19	101
18	98	20	24	77	121	6	21	101
21	100	21	30	79	156	9	21	135
15	101	27	27	75	160	12	26	133
18	110	21	28	75	161	10	35	140
Average						7.4	24.7	125.6

Lactic acid removed..... 27.5 mgm. per cent
Phosphocreatine-P resynthesized..... 10.3 mgm. per cent
Ratio 2.67

			STIMULATED 30 SECONDS RECOVERY 120 SECONDS					
16	87	20	19	54	143	3	33	123
17	92	12	16	63	108	−1	29	96
18	100	15	21	74	117	3	26	102
19	95	21	19	68	138	0	27	117
15	93	22	19	66	139	4	27	117
18	95	22	18	66	134	0	29	112
Average						1.5	28.5	111.2

Lactic acid removed..... 14.4 mgm. per cent
Phosphocreatine-P resynthesized..... 5.9 mgm. per cent
Ratio 2.44

lyzed during a single tetanus is resynthesized anaerobically afterward, and that this resynthesis takes place only during the first 20 or 30 seconds after the end of the tetanus.

In a previous paper (15b) it was stated that this anaerobic resynthesis, which Lundsgaard considers to be the immediate reaction in the recovery process, could be regarded rather as the restoration of pH equilibrium within the fiber, on the assumption that the pH of the frog muscle fiber is the same as that of the mammalian muscle fiber. Without abandoning that assumption it is evident that the explanation given above is independent of the exact pH of the fiber; it requires only that the pH be within the rather wide range over which the hydrolysis of phosphocreatine liberates base.

Since only 30 per cent of the hydrolyzed phosphocreatine can be resynthesized anaerobically, Lundsgaard considers that the remaining, larger, part must wait for oxidative reactions to furnish energy for its resynthesis. The finding that lactic acid removal is the factor controlling phosphocreatine resynthesis accounts for this limitation on the anaerobic process. This also makes it advisable to re-examine the experimental basis for Lundsgaard's hypothesis. If the immediate source of the energy for contraction is the hydrolysis of phosphocreatine, and if recovery consists of the resynthesis thereof, then the muscle must be able to perform this resynthesis at a sufficiently rapid rate to meet the demands of the contraction process. It was found previously (15b) that rabbit muscles produced about 60 mgm. per cent of lactic acid in a 5 second tetanus. From the value of approximately 180 cals. per gram for the heat of formation of lactic acid from glycogen, and approximately 400 cals. per gram of P for the heat of hydrolysis of phosphocreatine, determined in Meyerhof's laboratory (10), the formation of this 60 mgm. per cent of lactic acid should yield sufficient energy for the resynthesis of 27 mgm. per cent of phosphocreatine-P, or at the rate of about 325 mgm. per cent per minute. This is more than 30 times the rate actually found during the first minute of recovery, and more than 50 times the rate found in the second minute. In other words, the Lundsgaard hypothesis requires that the "recovery" process take place with enormously greater velocity *during contraction* than that at which it actually takes place *during recovery*. The data showing the anaerobic resynthesis of phosphocreatine in isolated frog muscles lead to the same conclusion. If 30 per cent of the "recovery" process after a 5-second tetanus requires 20 seconds (Nachmansohn) or 30 seconds (Lundsgaard) then the muscle must recover 13 or 20 times as rapidly during contraction as it is found to do afterward. As for oxidative resynthesis of phosphocreatine, Meyerhof and Nachmansohn (12) found that this is much slower in frog muscle than is the anaerobic resynthesis.

Lundsgaard (6b) found that iodoacetate muscles were capable of per-

forming much more work in oxygen than in nitrogen, at the expense of less *net* phosphocreatine breakdown. Mawson (9) has extended this work and has shown that with added lactate and oxygen very much more work was done than without lactate, and that lactic acid disappeared, probably by oxidation, under these conditions. It is *assumed* that phosphocreatine is hydrolyzed, then resynthesized by the oxidation of some lactic acid, for Lundsgaard has shown (6c) that there is no anaerobic resynthesis of phosphocreatine in iodoacetate muscles. *However, neither investigator has demonstrated that phosphocreatine actually is hydrolyzed during the contraction in oxygen.* The experimental procedure used by Lundsgaard was to stimulate companion iodoacetate muscles, one in oxygen, one in nitrogen, with single twitches at such a rate that the oxygen muscle was able to recover its oxygen by inward diffusion between two successive twitches. Then, when the nitrogen muscle had become non-irritable they were both analyzed for phosphocreatine content. Mawson's finding that lactate and oxygen are unable to prevent the rigor caused by increased carbon dioxide concentration makes it highly improbable that the iodoacetate muscle is capable of even oxidative resynthesis of phosphocreatine.

When it is considered that in iodoacetate muscles phosphocreatine is converted directly to hexosephosphates, the possibility that the hydrolysis of this substance can be the normal source of energy for contraction becomes even more remote. The present data show that the reconversion of hexosephosphate-P to phosphocreatine-P is very slow, relative to the resynthesis of that which has merely been hydrolyzed.

The theory of the sources of energy for muscular contraction presented in the first paper of this series makes it unnecessary to ascribe to phosphocreatine any functions incompatible with these data. Under anaerobic conditions, the normal muscle has two sources of energy available: the formation of lactic acid from glycogen, and the formation of hexosephosphate from glycogen and phosphocreatine. Hydrolysis of phosphocreatine occurs only in such measure as will prevent any change of pH within the muscle fiber. In the presence of adequate oxygen supply, lactic acid is oxidized. In iodoacetate muscles contracting anaerobically, the formation of lactic acid is inhibited, and the formation of hexosephosphate becomes the only source of energy. On this basis the correlation between heat production and phosphocreatine disappearance found in Meyerhof's laboratory (11) is easily understandable.

The work of Clark and his collaborators (1) on the frog heart has led to similar conclusions. They find that under anaerobic conditions lactic acid is formed; the phosphocreatine content, which is low to begin with, falls off very rapidly, and the lactic acid first formed passes into the surrounding Ringer's solution in neutral form. Activity continues until, in the presence of alkaline buffers, lactic acid precursors are exhausted; or, in

the absence of buffers, until the acidity reaches the point at which lactic acid formation is inhibited. Iodoacetic acid, they find, "is exerting a highly specific effect on some process or structure which is required only during anaerobic activity," presumably lactic acid formation.

Margaria, Edwards and Dill (7) also arrive at the same conclusions concerning the rôles of lactic acid and oxygen in muscular exercise in man. However, the present data furnish only partial confirmation of their interpretation of the "alactacid" oxygen debt. They find that the portion of the total oxygen debt which is rapidly paid is not due to lactic acid, and may be due to phosphocreatine hydrolysis. Such an interpretation is easily possible from the present data. However, that portion due to hexosephosphate formation is evidently paid very slowly, at least in the early part of the recovery period during which hydrolyzed phosphocreatine is being restored. Nothing in their data can be found to represent this portion of the "alactacid" oxygen debt, although it may be as important quantitatively as the part due simply to hydrolysis.

SUMMARY AND CONCLUSIONS

1. The early stages in the recovery process after muscular contraction have been studied in the muscles of cats and rabbits.

2. Lactic acid removal and phosphocreatine resynthesis are found to proceed *pari passu* in the early stages of recovery.

3. The amount of phosphocreatine resynthesized in a given time is found to be equivalent, in terms of base removed, to the amount of lactic acid concurrently removed.

4. The resynthesis of phosphocreatine from its hydrolysis products after contraction is found to proceed at a rate only a small fraction of that required by the Lundsgaard hypothesis.

5. The reconversion of hexosephosphate-P to phosphocreatine-P is found to be quite slow during the early part of the recovery period.

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CALORIGENIC ACTION OF FAT AND CARBOHYDRATE IN PANCREATIC DIABETES

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Received for publication February 23, 1935

The calorigenic action of fat has been explained as due to fat plethora (see Lusk, 1928). In diabetes, there is a high blood fat so that this fat plethora may be responsible for all or at least part of the increased metabolism of diabetes. The abundant ingestion of fat by the dog, as has been shown by Rubner (1902) and by Murlin and Lusk (1915), may produce a maximal increase in metabolism amounting to about 30 per cent of the basal level—a figure which is not far from the 40 per cent increase in metabolism which occurs as a result of diabetes in the cat (see Ring and Hampel, 1932). The questions of present interest were: Could fat ingestion by the normal cat increase metabolism by 40 per cent, and if so, would the blood fat reach a level similar to that found in diabetes? Would the respiratory quotient in normal animals after fat ingestion go below 0.75, which is common in diabetic cats?

It soon became apparent that cats were quite different from dogs in regard to the calorigenic effect of fat. Even when twice their daily caloric requirement was given in the form of fat, the metabolism was never increased more than 10 per cent, the respiratory quotient was scarcely lowered at all, and the blood fat was affected to only a minor degree. Arguing that a diminution in carbohydrate metabolism might be essential to increase fat utilization, I studied the effect of removal of the tail of the pancreas and a large part of the head. This procedure does increase the calorigenic action of fat.

With these partially depancreatized animals available, it seemed worth while to study the specific dynamic effect of carbohydrate as well.

METHOD. *Measurement of metabolism.* In measuring the calorigenic action of fat, one should have a continuous record of metabolism over a period of several hours. The apparatus was therefore arranged so that a small portion of the air leaving the box was continuously drawn into a bottle to occupy the space left by mercury which was flowing out at a constant rate. This air was later removed for analysis. Then by determining the rate of ventilation of the box, one could calculate the mean metabolism for the period. The ventilation was maintained at a rate

which caused the outgoing air to contain about 1.0 per cent carbon dioxide. The cat box rested on a platform supported by four springs. A lever attached to one end of this box recorded any movements of the animal. Usually these were so slight that they had a negligible effect on metabolism, but occasionally a period had to be discarded.

It was originally planned to measure the basal metabolism one day and the specific dynamic effect a few days later. Of course, slight differences in basal metabolism were expected on different days, but I felt that these would be of little importance in comparison with the large increase in metabolism to be expected after fat feeding. Experiments, however, showed that the effect of ingestion of large quantities of fat was very small in normal cats and usually within the range of variation of normal metabolism. Thus it became necessary to measure the basal metabolism and the specific dynamic effect on the same day. The final plan of an experiment was this. Cats were put into the respiration chamber at 9:00 a.m. and the compressed air was turned on. At 10:30, the cats had usually been quiet for at least half an hour and the outgoing air had reached a constant composition. Then three periods of one-half hour each were used to determine basal metabolism. After this the food, whose specific dynamic effect was to be studied, was given by stomach tube. The animal was allowed a half-hour in which to move about in a room and then returned to the box where the metabolism was measured during the second, third and fourth hours after the ingestion of the food. Previous studies had shown that a plateau of maximal metabolism was reached at this time. The above procedure has been modified by measuring the basal metabolism continuously for a period of five hours. The results, however, are constant throughout this period.

In the beginning, butter was used as the fat, but along with other refinements it seemed better to use a pure substance. Since oleic acid is easily obtained and will pass readily through a stomach tube, I continued the studies with this material. Of course, this is not a complete fat, but the results which I obtained were so similar to those found with butter that I believe the glycerine portion of the fat has very little to do with the specific dynamic effect. The oleic acid is for the most part supplied with glycerine and synthesized to neutral fat in the mucosa of the gastro-intestinal tract (see Munk, 1880; Munk and Rosenstein, 1891; Moore, 1903). The small amount of oleic acid which may reach the blood possibly upsets the acid-base equilibrium slightly, causing a blowing-off of carbon dioxide and thus a respiratory quotient which is too high. One would, however, expect that during the period of measurement the conditions within the body would be stable and the amount of fat (fatty acid) entering the blood would be equal to that leaving.

Partial pancreatectomy. If the tail of the pancreas and about three-

fourths of the head are removed, cats will usually digest food satisfactorily and will maintain weight without the use of insulin. Some will show a glycosuria, while others will not. The operation mentioned above is one spoken of as partial pancreatectomy.

RESULTS. In table 1 are given a few typical results of fat or fatty acid ingestion in normal cats. Each of the figures is the average of at least two measurements of metabolism and in most cases three or four. It seemed unnecessary to include the original figures because they checked so closely. It will be seen that, after the ingestion of oleic acid by normal cats, the metabolism scarcely changed except in cat 4, where the increase amounted to 10 per cent. The mean increase for the observations in this table is 2 per cent. Chambers and Lusk (1930) gave 10 grams of lard to dogs (about one-tenth the dose per kilogram which I gave cats), and obtained a mean increase of 3½ per cent during the third, fourth and fifth hours after

TABLE 1

Metabolism of normal cats before and after fat feeding

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER FAT (FATTY ACID) INGESTION		
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour
1	3.15	0.75	28.3	40—oleic acid	0.74	28.6
1	3.20	0.76	29.9	40—butter	0.78	29.3
2	2.40	0.79	27.7	35—oleic acid	0.75	28.0
3	3.50	0.78	31.0	40—oleic acid	0.78	31.7
4	3.55	0.75	31.2	35—oleic acid	0.74	34.3

ingestion. Murlin and Lusk (1915) gave fat to dogs in amounts which more nearly correspond to mine and found an increase in metabolism amounting to as much as 30 per cent. Magnus-Levy (1894) observed during the seventh hour, after 210 grams of butter had been eaten by human subjects, a maximal increase of 9 to 14 per cent. Hiller, Linder, Lundsgaard and Van Slyke (1924) noted that 1 gram of fat per kgm. given to man caused a rise in basal metabolism which varied between 0 and 15.5 per cent, with the average at 8.5 per cent. All these observations lead one to believe that the dog and man show a greater calorigenic action of fat than does the cat.

If plethora is responsible for the calorigenic action of fat, there should be some correlation between blood fat and metabolism. Hiller, Linder, Lundsgaard and Van Slyke (1924) paralleled their calorigenic measurements with determinations of blood fatty acids. In their subjects, one showed a decrease in blood fatty acids, one an increase of 80 per cent, and four an increase between 1.3 and 29 per cent. Man and Gildea (1932)

gave 3.5 to 4 grams per kgm. to men and found the increase in fatty acids of the blood averaged 62 per cent. Bloor (1914) obtained an increase of about 100 per cent in the blood fat of three out of four of his dogs after a large fat meal. These latter results on man and the dog are in contrast to those obtained on 4 cats where the maximal increases were 8, 10, 22 and 23 per cent (unpublished), after giving about 10 grams of oleic acid per kgm. These observations harmonize with the fact that the metabolism in most normal cats is practically unchanged as a result of fat ingestion.

TABLE 2

Metabolism of partially depancreatized cats after fat ingestion

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER OLEIC ACID INGESTION			PERCENTAGE INCREASE OR DECREASE IN METABOLISM
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour	
2	3.25	0.79	27.6	40	0.77	32.7	18
5	3.65	0.77	31.0	40	0.76	37.4	21
5	3.75	0.78	31.0	40	0.74	38.0	23
5	4.00	0.81	32.9	30	0.80	36.3	10
5	4.00	0.79	35.5	35	0.83	34.6	−3
4	3.75	0.77	34.4	40	0.76	38.8	13
4	3.65	0.79	34.9	40	0.76	38.2	10
4	3.85	0.84	34.8	40	0.76	40.1	15
4	4.10	0.76	46.6	40	0.80	45.1	−3
6	3.80	0.71	30.0	35	0.74	34.9	16
6	4.25	0.77	32.0	20	0.75	37.2	16
6	4.30	0.77	32.6	40	0.74	37.5	15
6	4.20	0.82	29.5	40	0.74	34.7	18
6	3.45	0.78	29.4	40	0.77	33.1	13
6	3.55	0.73	29.6	30	0.77	33.7	14
6	3.70	0.84	34.9	40	0.82	36.6	5
6	3.65	0.77	44.3	40	0.77	43.2	−2
7	4.15	0.80	24.5	40	0.78	27.9	14
7	3.65	0.70	37.1	40	0.72	35.9	−3

After partial removal of the pancreas, if the basal heat production stays about normal, the calorigenic action of fat is large—usually raising the metabolism by 15 per cent or more (see table 2). If, on the other hand, the basal metabolism is high, then fatty acid ingestion does not increase the metabolism further. In fact, the results are frequently lowered by 2 or 3 per cent. With intermediate basal metabolism the increases are frequently between these extremes.

The respiratory quotients are affected very little as a result of ingesting fatty acid. Five experiments showed an increase and thirteen a decrease. Of the five raised respiratory quotients, three were in animals having a high

metabolism and showing no specific dynamic effect. Had neutral fat been used instead of a fatty acid, the respiratory quotients might have shown a more constant and striking decrease after the ingestion. The results of the few experiments in which butter was fed were not noticeably different from those recorded in the tables.

Table 3 shows the specific dynamic effect of glucose in partially depancreatized cats. In most cases the caloric increase is not greater than 10 per cent. The respiratory quotients in all but cat 7 rose about 0.10, which is similar to results with the normal animals studied. This test would indicate that the capacity for burning sugar in most of these animals is not measurably decreased. In cat 7, where the respiratory quotient was low and was not raised by sugar, there was no specific dynamic effect. These results are included to show that in most cats studied the carbohydrate metabolism was not markedly upset though the calorigenic action of fat was greatly increased.

TABLE 3

Metabolism of partially depancreatized cats after glucose

CAT NUMBER	WEIGHT, KGM.	POSTABSORPTIVE		AFTER GLUCOSE INGESTION			PERCENTAGE INCREASE OR DECREASE IN METABOLISM
		R. Q.	Calories per square meter per hour	Amount, grams	R. Q.	Calories per square meter per hour	
5	3.80	0.79	31.7	20	0.90	34.9	10
5	3.75	0.78	27.2	7	0.86	30.0	10
6	3.35	0.77	31.0	7	0.88	32.4	5
2	3.30	0.81	28.8	20	0.89	33.7	17
4	3.70	0.79	39.1	20	0.89	39.9	2
7	3.60	0.72	46.5	7	0.72	43.9	−6

DISCUSSION. The removal of a large part of the pancreas would be expected to decrease the pancreatic lipase entering the intestine and for this reason the fat absorption. This effect, however, is not large. Cruickshank (1915) showed that partially depancreatized dogs absorbed 72.12 per cent of the fat from a diet low in fat. Pratt (1916), also using dogs, stated: "When a bit of pancreas measuring 1 cm. in size was left attached to the open main duct, there was only a slight disturbance of absorption. A test made nearly six months after the operation and three weeks before the death of the animal showed that 75 per cent of the nitrogen and 66 per cent of the fat were absorbed." In the cats used, I have left more pancreas than Pratt, so that the derangement in digestion is perhaps very slight. Nevertheless, there is probably some decreased ability to utilize fat, and thus the calorigenic effect per unit amount of fat absorbed in the depan-

creatized animals is even greater than the comparison with normal cats would indicate.

The large calorigenic action of fat disappears whenever the operated animals chance to show a basal metabolism as high as that in diabetes. This fact, together with the greater calorigenic action of fat in partially depancreatized cats having normal basal metabolism, shows that there is a derangement of the fat catabolism of the diabetic animal.

The specific dynamic action of glucose also disappears with the high metabolism of diabetes—a condition to be expected when there is large excretion of sugar taking place even before glucose is given.

CONCLUSION

1. The maximal calorigenic effect of ingestion of large amounts of oleic acid by normal cats is insignificant (see table 1).

2. In partially depancreatized cats with normal basal metabolism ingestion of oleic acid produces a far greater effect than in normal animals (see table 2).

3. If the basal metabolism of partially depancreatized cats is high, the specific dynamic effect disappears (see table 2).

4. Most partially depancreatized cats show the same rise in respiratory quotient as that following glucose ingestion in normal animals (see table 3).

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DYNAMIC CONSIDERATIONS ON THE RELATION BETWEEN AORTIC AND VENTRICULAR PRESSURE CURVES

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Received for publication November 9, 1934

It is generally held that the work done by the heart can be roughly calculated by the formula

$$W = QR + \frac{MV^2}{2g}.$$

The work (W) is the product of the volume of cardiac output (Q) times the blood pressure (R). To this is added the work necessary to accelerate the blood to the aortic velocity. This depends upon the mass of blood pumped (M) and the square of the velocity to which it is accelerated (V^2). In contrast to earlier preconceptions, Evans (1) shows clearly that the kinetic term $\frac{(MV^2)}{2g}$ is not to be ignored. Thus in a certain heart-lung preparation he shows that if the cardiac output is 0.67 liter per minute the kinetic fraction of the total cardiac work is 14 per cent; if the output is 1 liter per minute the kinetic fraction which increases geometrically is 25 per cent and if the output is increased to 1.7 liter per minute the work done in accelerating the blood reaches the remarkable figure of 50 per cent of the total work.

Evans calculated his results by taking mean velocity and pressure values. Katz (23) (working with the excised turtle heart) has shown that if these values are integrated the kinetic work is a still greater proportion of the total work. It should be pointed out, moreover, that 1.7 liter per minute is a rather small cardiac output for a normal resting dog and that figures well above these are to be found in the literature (2, 3, 4, 6) so that the percentages mentioned by Evans are not excessive.

Not only is the velocity factor recognized as playing an important rôle in the calculation of cardiac work, but it is also taken fully into account in discussions of the peripheral blood pressure. Thus the fact that the femoral pressure exceeds the carotid is shown to be due to the conversion of velocity into pressure energy (24, 25) and similar principles are used in discussions of the Korotkow sounds (26). Many other authors make use

of these concepts in discussing arterial pressure problems (cf. 27) but the above may suffice as examples.

An interesting paradox becomes evident when one contrasts this widely held point of view with the generally accepted picture of the relation between aortic and left ventricular pressure curves during cardiac systole. They are usually pictured parallel and at about the same height. This, however, leaves no room for the kinetic term which we have just discussed. The energy to accelerate the blood can only come from the intra-ventricular pressure and this quantity must rise above the aortic pressure whenever the aortic velocity rises above zero. Furthermore its excess above the aortic pressure must always be proportional to the amount of kinetic work the heart is doing.

Wiggers (5, 6, 7) and Piper (8), however, have observed pressure curves of this type and, since they occurred with high frequency in records that seemed technically sound, these authors were at one time inclined to regard them as expressing true pressure conditions. We hope here to substantiate this earlier view and to give a quantitative account of the meaning of this pressure difference. The more conventional relationship, however, appears in most treatises (5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 27).

The difficulties in getting records where the ventricular pressure exceeds the aortic may be twofold.

Most important is the fact that the opening of the chest and other manipulations necessary to take intracardiac pressures in the usual manner may reduce the stroke volume to $\frac{1}{3}$ of its preoperative value with no appreciable change in blood pressure (21). The barbituric anesthetics have a similar effect (6), and when the dog's blood pressure reaches low values such as 120/80 (mean 95 mm. Hg), the stroke volume may be $\frac{1}{6}$ normal (21). The low stroke volume of course reduces the kinetic factor and the excess ventricular pressure is minimized. Furthermore, the most convenient place for entering the exposed ventricle is just below the aortic orifice (5). At this part of the ventricle the velocity is nearly as great as that in the aorta, and the energy which exists as pressure at the apex has been changed to energy of motion at this part of the ventricle. In some of the earlier work on unoperated animals (12, 13, 17) the heart was entered through the aorta or one of its branches and the cannula was stopped just below the aortic valves, that is, in a region of high velocity. Here again a fraction of the ventricular pressure has been changed to kinetic energy and the aortic and ventricular pressure curves should be parallel.

EXPERIMENTAL. The pressure curves which we present here belong to the same series as those used to illustrate a previous communication (4). They were recorded by means of a new hypodermic manometer (4) of high natural frequency whose cannula is an ordinary Luer needle.

It can be used in the unoperated animal with only a local anesthetic.

All the complications of surgical operation and shock are avoided and it is possible to take simultaneous records from the apex of the ventricle and from the proximal carotid artery in an animal which is completely normal and unanesthetized.

In figure 1 there is a series of intraventricular pressure curves taken from the apex and simultaneously with a series of carotid pressure curves. The ventricular curves exceed the carotid by varying amounts. This variation is caused either by variation in the stroke volume due to respiration or by respiratory variation in the position of the intraventricular needle.

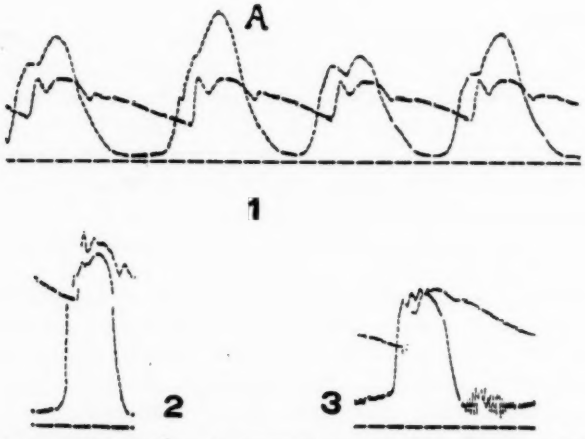

Fig. 1. Intracardiac and aortic pressure curves taken simultaneously from a normal unanesthetized and unoperated dog. Time 1/20 second.

Fig. 2. Same taken from open chest preparation in poor condition. Ventricular pressure slightly higher than aortic. The two manometers differ somewhat in sensitivity.

Fig. 3. Same. Aortic regurgitation. Ventricular needle at mouth of aorta. Closed chest.

In figure 2 there is little or no excess ventricular pressure because the chest was opened and the animal in poor condition, though the blood pressure was high (adrenalin). The ventricular curve was taken from near the apex but the heart was not doing much kinetic work.

In figure 3 there is no excess ventricular pressure but the reason is entirely different from the preceding case. The pressures were recorded under local anesthetic and the chest was unopened. The animal was suffering from chronic experimental aortic regurgitation (2). The intraventricular needle was evidently in the base of the ventricle near the aortic orifice, because the turbulence which caused the diastolic murmurs impinged

directly upon the orifice of the needle and caused violent fluctuations of pressure within the manometer. With the intraventricular needle thus known to be in a region of high systolic velocity it is not surprising that the aortic and ventricular curves have summits that are essentially parallel.

Quantitative considerations. We were next interested to see whether a quantitative estimation of the aortic velocity, the volume output and the

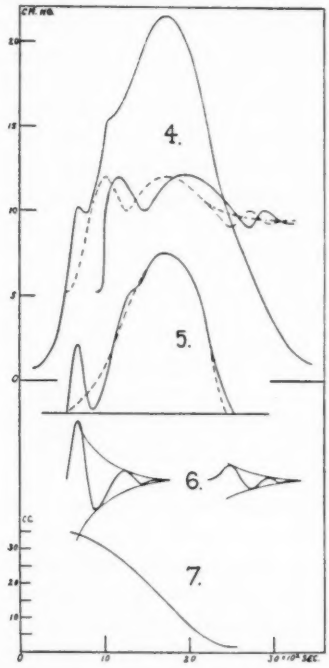

Fig. 4. Figure 1A. Plotted to one scale.

Fig. 5. Ventricular pressure minus aortic pressure.

Fig. 6. Transient pressure waves substracted from figure 5 and giving the dotted curve in figure 5.

Fig. 7. Left ventricular cardiac blood volume during ejection, assuming ½ sq. cm. area of aorta, 35 cc. total stroke volume.

ventricular emptying curve can be calculated from the values of simultaneous carotid and ventricular pressure curves.

In figure 4, the values of figure 1A have been plotted to scale. The full line curves show the pressure observed in the ventricle and carotid. Where one is dealing with a steady state and rigidly bound system, such a set of pressure data would enable one immediately to compute the velocity of the liquid.

Since, in our case, sufficient data are not available to permit a rigorous analysis, we shall make certain arbitrary assumptions. While these assumptions will leave the numerical evaluations subject to some uncertainty, it will be possible to draw conclusions from these calculations which will be definitely outside the range of uncertainty. If the contributions to the pressure differences due to time of pressure propagation and transient changes can be corrected for, we may then apply Bernouilli's principle to the evaluation of velocities.

The apex of the ventricle and the lumen of the aorta during ejection can be regarded for our purposes as parts of the same blood conducting tube. In the apex of the ventricle the velocity of flow can be regarded as zero, and that in the aorta can be regarded as maximal. Neglecting the slight losses due to friction, the total energy according to Bernouilli's principle is identical in the two parts of the system. Thus in the ventricular apex the pressure is high and the velocity low (zero) and in the aorta the pressure is lower and the velocity high. The relationship can be expressed by the formula

$$P_a + \frac{V_a^2}{2g} = P_v + \frac{V_v^2}{2g} \qquad \text{(II)}$$

Where P_a, V_a, P_v and V_v are pressures and velocities in the ventricle and aorta respectively. Letting $V_v = O$ the formula for calculating aortic velocity becomes

$$V_a^2 = 2g\,(P_v - P_a) \qquad \text{(III)}$$

Two difficulties present themselves in applying these principles to the blood stream. First, since the walls of the system are semi-elastic, the pressure changes would be transmitted through the system with a velocity which depends upon the pressure values (even through energy loss to the walls is neglected).

Second, since there is a marked departure from steady state, momental considerations arise which somewhat modify the values obtained for the velocities. Such corrections, however would be small and cannot readily be evaluated.

It will be assumed that the pressure is propagated from one point of observation to the other in 2/100 of a second when the pressure observed is 100 mm.; second, that the propagation rate is linearly dependent upon the pressure (22). Thus, at 50 mm., 4/100 second is required. In other words, if the distance from one observation point to the other is 8 cm., the velocity of propagation at 100 mm. pressure is 4 meters per second.

The effect of the rapid rise in the propagation rate with pressure is of

great importance in connection with the transient effects which follow the initial pulse. At the initial valve opening, the first sharp pressure rise is transferred slowly down the aorta. However, by the time that the pressure has risen to twice the value, the pressure pulse is now traveling according to our assumption with twice the velocity. Thus the latter part of the pulse almost overtakes the first part, causing a much sharper pressure rise in the carotid than took place in the ventricle. The branching of the aorta creates a partial obstruction to the blood flow which is aggravated by the introduction of the cannula. This obstruction results in a reflection of the pressure pulse back to the ventricle, thus giving rise to a highly damped standing wave or transient.

The dotted curve shows the displaced values at the carotid as they appear, corrected for the time elapsed for pressure propagation.[1] The full line curve in figure 5 shows the difference in pressures when corrected for propagation time. It is fairly evident that a transient is superimposed on the general pressure change. If we assume a rather simple slow pressure change as indicated by the dotted curve, we have a residue of a typically damped disturbance which dies out just before the pressure difference reaches a maximum, as shown in figure 6.

While it is not possible to determine the extent of velocity disturbances which arise in connection with this transient, a computation of velocity (cf. 20) on the basis of the smoothed underlying pressure change cannot be greatly in error. When the pressure falls, due to the momentum of the blood leaving the heart, it falls below the value which would have been maintained if relatively steady flow conditions were realized. Furthermore, a reflection of the higher pressure condition from the carotid causes a subsequent pressure rise after the closing of the valve. Thus a transient and also highly damped standing wave is set up in the aorta which precedes the establishment of equilibrium.

In figure 4, the dash-dot curve has been drawn to indicate a probable value of the pressure corrected for this transient disturbance, the pressure values of the transient being evaluated as before in figure 5. Since the disturbance arises from a much less violent pressure change, the amplitude is much smaller than the initial transient but dies out in about the same length of time. The period of this disturbance, however, is less, due to the closure of the valve which shortens the path and creates a closed end tube. In evaluating the velocities of the blood leaving the ventricle, the higher values of pressure difference are more nearly correct than those of the steady state because of the persistence of the velocities due to momentum.

[1] We consider that in using a branch of the aorta we are measuring a pressure contour similar to that in the aorta itself. Perhaps it is identical in its major quantitative features, when the "transients" (see below) have been eliminated.

In this discussion we have attempted to bring out first the necessity of correcting for time of pressure propagation and second the influence of transients on the pressure changes observed. By a somewhat arbitrary procedure, a corrected pressure difference curve has been obtained which cannot be in error by more than a small percentage. By application of Bernoulli's principle (table 1, cols. 1, 2, 3), we obtain values for velocity of the blood stream leaving the heart (presented in columns 4, 5).[2] If we assume the cross area of the aorta to be 0.5 sq. cm., these data enable us to compute the volume of blood leaving the heart (column 6). Since this value for the volume agrees roughly with those obtained by other methods, the pressure differences exhibited must be of the right order of magnitude to bring about the purging of the heart.

Looking at the problem from an energy standpoint, work is done by the heart in expelling blood. A part of this work is spent in giving kinetic energy to the blood, i.e., energy due to its velocity; a part is spent in turbulence and transient disturbances; and a part is stored up in potential energy in the expanded arterial wall. From this standpoint, it is clear that the pressure differences arising in the heart must exceed those in the aorta in order to give the blood sufficient velocity to leave the heart in the allotted time. Since the volume of blood arrived at from our velocity considerations is the right order of magnitude, the pressures indicated by the manometers must not be regarded as anomalously high, but as absolutely essential to the mechanical process of purging the heart. If these computations are in error, they err on the side of requiring too small rather than too high a pressure difference. Since we have assumed that practically all except the first initial sharp rise in pressure is transformed into kinetic energy of the blood, we have placed a minimum requirement on the pressure difference. To whatever extent the pressure energy is lost in turbulence or potential energy of the aorta walls, to that extent the veloci-

[2] The velocity figures which we have given below are the inevitable conclusion from the pressure differences in the experimental record. If the cross area of the aorta is assumed to be 0.5 cm.^{2} the total stroke volume comes out 35 odd cc. A larger cross area necessitates a proportionately larger stroke volume.

It should be noticed that the systoles immediately following and preceding the one analyzed in detail give evidence (see fig. 1) of much smaller velocities and hence of much smaller stroke volumes. For theoretical reasons the systole giving the greatest pressure difference is the one that should be taken as an example, the average systole probably gives rise to smaller velocities.

The fact that the femoral systolic pressure exceeds that in the brachial artery by 20–100 mm. Hg (25) might be taken to indicate that the velocity in the transverse aorta exceeds that in the iliac by figures of the same order as those which we attribute to the velocity in the aorta.

The fact that the blood velocity is greater than the pulse wave velocity is paradoxical. Of course the high velocities do not obtain out in the arterial tree—only in the aorta, and there only before the walls have taken up blood by elastic recoil.

ties must be reduced, thus corresponding to a smaller volume output for the heart.

It should perhaps be pointed out that all the above considerations are based upon the assumption that the normal movement of blood in the blood vessels is irritational, i.e., streamline. With this type of motion, pressure energy produces flow most economically, the work done being proportional to the square of the velocity imparted to the blood.

If, on the other hand, the stream of blood leaving the heart is turbulent,

TABLE 1

Aortic velocity and ventricular volume changes as calculated from the differences between aortic and ventricular pressure curves

TIME	$P_v - P_a$	$2g(P_v - P_a)$	AORTIC VELOCITY	AVERAGE DISTANCE TRAVELED BY PARTICLE OF AORTIC BLOOD	LEFT VENTRICULAR RESIDUE ON BASIS OF 35.93 CC. TOTAL EJECTION
sec.	*cm.* Hg	*dynes/cm.²*	*cm./sec.*	*cm.*	*cc.*
0.06	0.11	2932.2	54.2	0.54	34.73
0.07	0.56	14927.4	122.2	1.22	34.12
0.08	1.21	32253.8	179.6	1.80	33.22
0.09	2.02	53845.1	232.0	2.32	32.06
0.10	2.92	77835.5	279.0	2.79	30.77
0.11	3.93	104758.1	323.7	3.24	29.05
0.12	5.10	135945.6	368.7	3.69	27.20
0.13	6.50	173264.0	416.2	4.16	25.12
0.14	7.60	202585.6	450.1	4.50	22.87
0.15	8.46	225509.8	474.9	4.75	20.50
0.16	9.11	242836.2	492.8	4.93	18.03
0.17	9.48	252698.9	502.7	5.03	15.57
0.18	9.40	250566.4	500.6	5.01	13.01
0.19	9.03	240603.7	490.5	4.91	10.56
0.20	8.31	221511.4	470.7	4.71	8.20
0.21	7.25	193256.0	439.6	4.40	6.00
0.22	5.40	143942.4	379.4	3.79	4.11
0.23	3.25	86632.0	294.3	2.94	2.64
0.24	1.57	41849.9	204.6	2.05	1.61
0.25	0.25	6664.0	81.6	0.82	1.20

there is an added loss which is also proportional to the velocity. When the blood leaves the heart quietly the aortic stream may not be turbulent, but whenever there are systolic murmurs or thrills, the aortic stream is probably turbulent. Under these conditions then the work which the heart must do to accelerate the blood is abnormally high.

It is to be hoped that the factors which we have attempted to bring out in a tentative and far from adequate analysis will emphasize the need for a further and far more rigid analysis of hemodynamic considerations in cardiac physiology and pathology.

SUMMARY

It is shown that the kinetic work done by the heart is accomplished because the intra-ventricular pressure markedly exceeds the aortic pressure during ejection.

Experimental curves of excess intra-ventricular pressure, when used to calculate the aortic velocity, the ventricular emptying curve, and the stroke volume, give results within reasonable limits.

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THE DIFFUSION OF POTASSIUM FROM RESTING SKELETAL MUSCLES FOLLOWING A REDUCTION IN THE BLOOD SUPPLY

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Received for publication February 7, 1935

Potassium is normally maintained in skeletal muscle at a much higher concentration than in the body fluids. There are, however, many conditions under which a loss of potassium from the muscles may occur: injury and death of muscles (Horton, 1930, etc.), perfusion or immersion in solutions low in potassium (Ernst and Takacs, 1931, etc.), increase in acidity in the surrounding fluid over that in the muscle (Netter, 1928; Fenn and Cobb, 1934), possibly severe fatigue (Mitchell and Wilson, 1922), etc. A loss of irritability is usually supposed to accompany a loss of potassium from skeletal muscles.

The following experiments indicate that potassium may diffuse out of resting mammalian skeletal muscle under conditions other than those reported above, namely, when the blood supply to the muscles is temporarily reduced and that this may occur without apparently any marked loss in irritability or damage to the muscles.

METHODS AND RESULTS. Cats were used for these experiments, anesthetized with urethane. The blood supply to the right hind leg was reduced by three methods: 1, by loss of blood due to continuous bleeding; 2, by clamping the abdominal aorta; 3, by stimulation of the lower abdominal sympathetic chain, thus causing vasoconstriction in the muscles of the leg. The venous blood was collected over a measured period of time into 15 cc. graduated centrifuge tubes by cannulation of the femoral vein, heparin having been given intravenously to prevent clotting. After centrifugalization, the total volume and the approximate level of the red cells were read from the centrifuge tubes to give an approximate measure of the concentration of the blood. The rate of blood flow from the femoral vein was calculated in cubic centimeters per minute. Blood samples which showed any visible trace of hemolysis after centrifuging were discarded. The potassium content of the plasma was determined, after precipitation of the proteins, by the method of Kramer and Tisdall (1921), or Kerr's (1926) modification of this and in a few cases by the method of Shohl and Bennett

(1928). Variations in the concentration of potassium in successive blood samples of less than 6 per cent were considered insignificant.

The procedure used in the different methods of reducing the blood flow and the results obtained were as follows. After discarding the first few cubic centimeters which flowed from the vein after inserting the cannula, 10 to 13 cc. were collected as the normal sample. In four experiments successive samples of a similar amount were then collected continuously until respiration ceased, each sample being analysed for potassium. These experiments served as controls for the other experiments and for determining the effect of a reduction in the blood supply due merely to progressive loss of blood. When the venous blood was allowed to flow continuously without any other procedure, the potassium content remained constant

TABLE 1

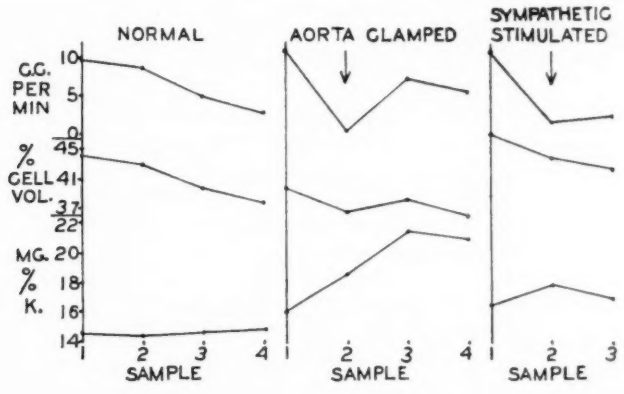
The effect of continuous bleeding on the potassium content of venous blood plasma
Milligrams per cent potassium and percentage variation from first normal sample

SAMPLE 1, NORMAL	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6	SAMPLE 7	SAMPLE 8
17.82	17.98		18.97	22.02	34.30		
	+0.89%		*+6.45%*	*+23.56%*	*+36.36%*	Cat dead	
12.50	11.83	12.23	12.35	13.79	20.03		
	−5.36%	*−2.16%*	*−1.20%*	*+10.32%*	*+60.24%*	Cat dead	
13.16	13.94	12.49	12.94	12.86	13.21	13.57	15.39
	+5.92%	*−5.10%*	*−1.64%*	*−2.30%*	+0.41%	*+3.13%*	*+16.91%*
13.92	13.82	13.26	13.78	14.98			
	−0.68%	*−4.74%*	*−0.97%*	*+7.60%*	Cat living		

within plus or minus six per cent of the first normal value until the last one or two samples just preceding the death of the animal when the potassium content rose rapidly, even reaching a value 60 per cent above normal. Table 1 gives the data for these four experiments, the absolute amount of potassium found in each successive sample and the percentage variation from the first normal sample being recorded. The average values for the amount of potassium, the rate of blood flow and the concentration of cells in the blood are plotted in graph 1a for the first four successive samples. The percentage of cells in the blood decreased progressively as did also the rate of flow.

In the second group of experiments a clamp was placed on the abdominal aorta during the collection of the second blood sample which varied from 5 to 23 minutes in the various experiments. The rate of blood flow through the hind leg was thus greatly reduced, the extent depending on the blood

pressure, the collateral circulation and the rate of dilution with tissue fluid. The potassium content of the plasma rose, usually markedly, in the blood collected during the period of clamping, varying from 2 to 42 per cent above normal with an average of 21.3 per cent, and continued to increase during the sample immediately following removal of the clamp (even if allowance is made for changes in the concentration of the blood) so that the potassium was then from 4 to 63 per cent above normal with an average of 44 per cent (table 2). The fourth sample showed a tendency in some cases to fall. During the clamping of the aorta, as shown by graph 1b (average of six experiments) the rate of flow and the percentage of cells in the blood decreased markedly while the potassium increased and during the following



Graph 1a Graph 1b Graph 1c

Graphs showing the plasma potassium content (lower curves), concentration of blood (middle curves) and rate of blood flow (upper curves) in successive samples of femoral venous blood during continuous bleeding with normal conditions (graph 1a), with clamping of artery (graph 1b), and with sympathetic stimulation causing vasoconstriction (graph 1c). (Cats—resting conditions.)

sample the rate of flow and the percentage of cells returned toward normal while the potassium continued to increase. In the fourth sample all three values fell.

In the third group of experiments the blood supply was reduced by vasoconstriction brought about by stimulation of the sympathetic chain during the collection of the second blood sample, which lasted from 4 to 14 minutes in the different experiments. The potassium content of the venous blood collected during the sympathetic stimulation again showed an increase varying from 2 to 22 per cent over the first normal value with an average increase of 9.2 per cent for the 15 experiments (table 3). The potassium content of the plasma continued to rise in four experiments and

decreased slightly in eight experiments during the third sample. The rate of flow decreased with the vasoconstriction but showed a slight increase in

TABLE 2

The effect of clamping the abdominal aorta on the potassium content of the blood plasma from the femoral vein

Milligrams per cent potassium and percentage increase over first normal sample

SAMPLE 1, NORMAL	SAMPLE 2, CLAMPED	PER CENT INCREASE OVER NORMAL	SAMPLE 3, AFTER	PER CENT INCREASE OVER NORMAL	SAMPLE 4, AFTER
11.54	14.17	+22.8			
	16.43	+42.3	18.06	+56.5	
16.04	16.39	+2.2	16.69	+4.0	
13.83	17.71	+28.1	16.85	+21.8	20.70
18.26	23.93	+31.1	29.72	+62.8	30.04
16.50	20.16	+22.2	23.45	+42.1	21.20
21.20	23.82	+12.4			
12.33	14.27	+15.7	18.07	+46.5	16.42
13.11	16.95	+29.3	19.96	+52.2	18.99
14.29	15.47	+8.3			
	16.47	+15.3			
14.88	15.93	+7.2	18.80	+26.3	17.85

TABLE 3

The effect of sympathetic stimulation on the potassium content of the blood plasma from the femoral vein. Muscles at rest and intact

Milligrams per cent potassium

BEFORE SYMPATHETIC STIMULATION	DURING SYMPATHETIC STIMULATION	PER CENT INCREASE WITH SYMPATHETIC STIMULATION	AFTER SYMPATHETIC STIMULATION
15.95	17.23	+8.0	15.63
14.55	17.23	+18.4	17.86
19.18	23.53	+22.7	16.61
20.59	20.94	+1.7	20.78
21.30	22.36	+5.0	
23.78	25.20	+6.0	
22.72	25.56	+12.5	
14.40	15.37	+6.7	15.42
15.73	17.57	+11.6	18.73
17.93	18.47	+3.0	18.28
16.30	17.97	+10.3	25.23
(19.57)	21.21	+7.1	20.21
19.57	20.90	+6.8	20.23
18.91	19.89	+5.2	19.69
14.62	16.43	+12.4	15.34

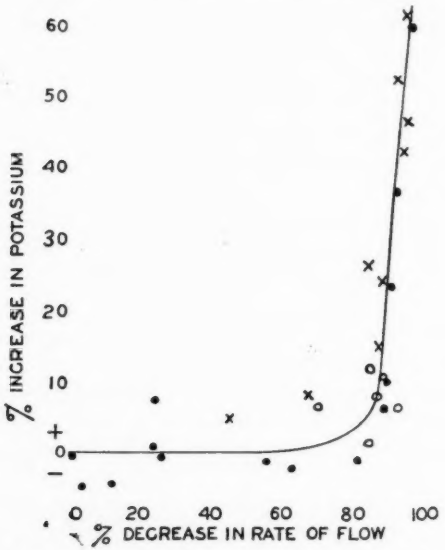
the third sample whereas the percentage of blood cells fell continuously as shown in graph 1c (average of four experiments).

In all of the above experiments the muscles were at rest. In five additional experiments blood samples were taken similar to this latter group but with the muscles contracting rhythmically throughout as a result of stimulation of the 6th and 7th anterior spinal roots. The potassium content of the venous blood plasma increased markedly (from 22 to 50 per cent above the normal value) in those blood samples collected during the period when the muscle reached the state of complete exhaustion no matter in which sample this occurred. This marked rise completely overshadowed any possible effect of sympathetic stimulation. As the blood pressure was already low due to the operative procedure, the loss of a very little blood resulted in a simultaneous slowing of the circulation and a rapid fall in the contractions. As the rate of flow was not measured in these experiments it is difficult to say how much of the increase in potassium could be attributed to the muscular exhaustion apart from that due to the reduction in the blood supply.

Discussion. The above experiments show that under conditions of a reduced blood supply the potassium content of the venous blood returning from the limb of a cat contains a greater amount of potassium in the plasma than was present previous to the reduction in blood flow. When the percentage increase in the potassium is plotted against the percentage decrease in the rate of venous flow a most interesting relationship is seen, as shown in graph 2. As the rate of flow is decreased the potassium concentration remains fairly constant within the limits of -5 to $+8$ per cent, or possibly increases slightly, until the rate of flow is decreased about 80 per cent, which is equivalent to a rate of blood flow in the femoral vein of about two to three cubic centimeters per minute. From this point on, further reduction causes a marked rise in the concentration of potassium in the venous blood. The graph shows that the potassium increases approximately 25 per cent with 90 per cent reduction in rate of flow and 60 per cent with 98 per cent reduction in rate of flow. This latter value corresponds to about 0.3 cc. per minute. This relationship occurs no matter whether the blood flow is reduced by hemorrhage, vasoconstriction or clamping of the artery. In the clamping experiments since the potassium continued to increase in the blood sample collected immediately after the clamping period this latter amount probably gives a more correct value of the maximum potassium increase resulting from a reduction in blood flow and is therefore used in plotting graph 2.

As changes in the concentration of the blood occurred during the reduction in the rate of flow, all of the values both for the increase in potassium and the rate of flow were recalculated making allowance for the dilution assuming that the potassium content of the diluting fluid was equal to that in normal blood (see below). This correction did not, however, alter the general relationship.

It is assumed that this sudden increase in potassium in the venous blood with a reduction in the rate of circulation is due to the liberation of potassium from the skeletal muscle fibers. It is not due to concentration of the blood since dilution always occurred. Nor is there reason to believe that the source of the potassium is the extracellular tissue fluid which dilutes the blood when the supply is reduced since 1, the increase in potassium is not proportional to the amount of dilution; and 2, analyses of



Graph 2. The effect of a reduction in the rate of blood flow through the resting hind limb of cats on the concentration of potassium in the blood plasma from the femoral vein.

●—normal bleeding.

○—sympathetic stimulation.

x—artery clamped (allowance is made for the delayed increase in potassium obtained in the sample following the clamped period).

lymph show no greater potassium content than that in blood serum (Meyer-Bisch and Gunther, 1925) and the salt content of lymph and tissue fluid are identical according to Drinker and Field (1933). No increase in serum potassium is reported by Kerr (1926) in dogs 24 hours after severe hemorrhage when marked dilution had occurred. Although samples of arterial blood were not analysed it seems hardly likely that clamping the abdominal aorta or stimulating the lower abdominal sympathetic chain peripheral to its section could cause a rapid increase in the potassium of the general circulating blood. The increase in potassium after continuous bleeding, however, may be due to a rise of potassium in the general circu-

lation and may be derived from tissues other than skeletal muscle. There is also no reason to believe that the potassium is derived from the corpuscles of the blood under conditions of slowed circulation and its accompanying change in oxygen and carbon dioxide tension since Doisy and Eaton (1921) have shown that variations in the carbon dioxide tension of the blood do not cause any shift of potassium between cells and plasma.

The most plausible explanation for the increase of potassium in the blood would be to assume that the diluting fluid is derived, beyond a certain point, from the potassium-rich intracellular muscle fluid, or that the loss of tissue fluid from the muscle beyond a certain critical point is the direct factor in causing the potassium in the muscle to be liberated rather than the reduction in blood supply itself. However, when the absolute or percentage increase in potassium is plotted against the extent of dilution no such clear relationship is seen as shown in graph 2 for the reduction in blood flow.

The potassium is, therefore, apparently liberated from the muscle suddenly when the blood supply is reduced below a certain critical level and the question arises whether this corresponds to a sudden decrease in the irritability of the muscle. To test this, three experiments exactly similar to the most severe clamping experiments were performed, except that the sciatic nerve was stimulated at intervals before, during and after the clamping of the aorta and the resulting isotonic contractions of the tibialis anticus were recorded. No marked loss of irritability as thus measured occurred with a temporary reduction in the rate of flow of 95 per cent over a period of time longer even than in those clamping experiments showing the most marked rise in potassium. It must be noted, however, that the increase in blood potassium in these experiments must represent only a comparatively small loss from the muscles considering the high concentration of potassium in the muscles and the number of muscles involved. However, complete loss of irritability in contracting muscles, brought about by rapidly repeated contractions accompanied by a reduction in blood flow, was always associated with a rise in potassium.

Without knowing what other changes occur in the muscle and blood at the time when the potassium begins to diffuse rapidly from the muscle, and without further experiments to determine if a reduction in the oxygen tension of the blood, without a reduction in the rate of flow with its accompanying dilution, causes a similar increase in plasma potassium, it is futile to discuss the significance of the results described above.

These experiments were started originally in an attempt to show whether or not sympathetic stimulation causes a liberation of potassium, because potassium had been shown to have an effect on skeletal muscle somewhat similar to that obtained upon stimulation of the sympathetic chain (Baetjer, 1934). When it was found, as described above, that sympathetic stimulation caused the liberation of potassium into the blood, the question

arose as to whether this was a direct result of the sympathetic stimulation or a secondary result due to the accompanying vasoconstriction. These experiments on the effect of reducing the blood supply to skeletal muscle were therefore undertaken. It can be seen from graph 2 that there is no evidence that sympathetic stimulation as such liberates potassium, as no more potassium was obtained in these experiments than could be accounted for by the reduction in blood flow. If heart muscle loses potassium in the same manner as described above for skeletal muscle one wonders if the increase in potassium obtained by Howell and Duke (1908) upon stimulation of the vagus nerve was perhaps due to the accompanying cessation of the circulation in the heart.

CONCLUSIONS

1. When the blood supply through the leg muscles of a cat was reduced, either by hemorrhage, vasoconstriction, or temporary blocking of the arterial supply, the potassium content of the venous blood plasma showed no significant increase unless the rate of flow in the femoral vein was reduced to about eighty per cent of its normal value. Beginning at this point further reduction produced a rapid and marked increase in the plasma potassium. This is attributed to a loss of potassium from the muscles.

A temporary reduction in the blood supply such as led to this marked rise in plasma potassium did not cause any marked decrease in the irritability of resting skeletal muscle as measured by the height of occasional isotonic contractions.

2. Stimulation of the lower abdominal sympathetic chain caused an increase in the potassium of the venous blood plasma but this was no greater than could be accounted for by the reduction in blood flow due to the accompanying vasoconstriction.

The author is indebted to the following persons for their assistance in making some of the chemical analyses reported above: Dr. N. D. Kehar, Dr. L. Kajdi, and Mr. William Fusting.

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THE EFFECT OF POTASSIUM (AND CALCIUM) ON THE CONTRACTIONS OF MAMMALIAN SKELETAL MUSCLE

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Received for publication February 7, 1935

A certain optimal concentration of potassium, equivalent to or slightly higher (Hegnauer, Fenn and Cobb, 1934) than that in Ringer's solution or blood is considered necessary for the normal irritability of skeletal muscle. An excess of potassium above this, according to the generally accepted view, exerts a depressant action on muscular excitability which may lead to complete paralysis. An excess of calcium chloride on the other hand is usually, although not always, reported to have a beneficial effect on skeletal muscle contractions in lower concentrations but a harmful effect in higher concentrations.

Practically all of the experiments on this subject reported in the literature have been made on frog muscles. The following experiments, which do not confirm these results, were made on mammalian skeletal muscles with intact circulation.

METHODS AND RESULTS. Cats under urethane anesthesia were used. Rhythmical break shocks from an induction coil at the rate of 120 per minute were applied to the 6th and 7th anterior roots, to the sciatic nerve or directly to the muscle, and the resulting isotonic contractions of the right tibialis anticus muscle were recorded. In some experiments blood pressure tracings were obtained from the carotid artery and in a few experiments plethysmograph records of the leg volume were also made. At intervals, while the muscle was contracting rhythmically, $1\frac{1}{2}$ to 2 cc. of the test solution were introduced, over a period of 5 to 15 seconds into the arterial blood stream supplying the right hind limb. This was accomplished through a cannula placed in the left common iliac artery close to the bifurcation of the aorta. For control two cubic centimeters of Ringer's solution with a potassium chloride content of 0.042 per cent were introduced at intervals so that any effect due to the addition of this small amount of fluid could be detected. All solutions were adjusted to a pH of approximately 7.4.

The results were uniform and definite. The introduction of two cubic centimeters of normal Ringer's solution was entirely without effect on the

height of the muscle contractions and on the blood pressure and produced only a very slight increase in leg volume (fig. 1a). When an excess of potassium chloride was added to the Ringer's solution so that the concentration of potassium chloride was 0.2 per cent or 0.4 per cent with or without a reduction in the sodium chloride content for isotonicity or when an isotonic 0.85 per cent potassium chloride solution was introduced, a marked temporary beneficial effect on the muscle resulted as indicated by a temporary increase in the height of the contractions (fig. 1b). These observations were repeated 38 times in a number of cats. A distinct improvement in the contractions occurred 32 times whereas no effect was ob-

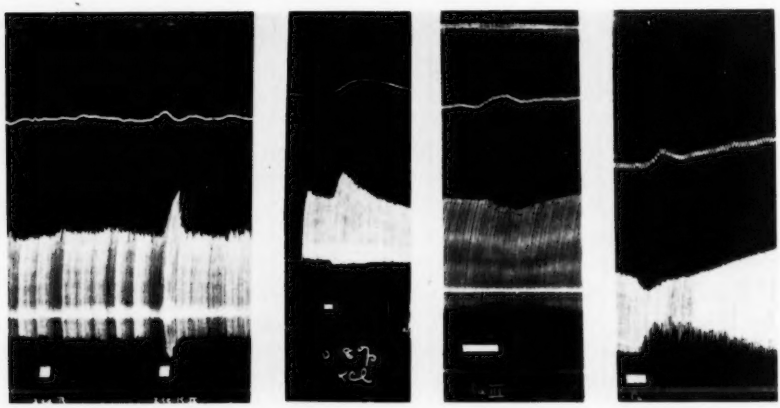

Fig. 1a 1b Fig. 2 Fig. 3 Fig. 4

The effect of normal Ringer's solution, excess KCl and excess $CaCl_2$ on the leg volume (upper curve), blood pressure (second curve), and muscle contractions induced by stimulation of the motor nerve.

1a: Ringer's solution with 0.042 per cent KCl; 1b: Ringer's solution with 0.4 per cent KCl; 2: Isotonic 0.8 per cent KCl solution (curarized muscle stimulated directly); 3 and 4: Ringer's solution with 0.6 per cent $CaCl_2$.

served 6 times. In these latter cases the contractions were falling rapidly in height and previous injections of potassium chloride had been made in rapid succession. The duration of the various phases of the response was as follows: latent period from beginning of injection of test fluid to beginning of rise in contractions, depending on the rate of injection, average 5 seconds, extremes 2 to 10 seconds; period of increasing contraction height, average 4 seconds, extremes 2 to 10 seconds; the maximal contraction height was maintained for only 2 or 3 contractions; period of decreasing contraction height, average 10 seconds, extremes 2 to 26 seconds. Usually the contractions returned to the level preceding the introduction of potas-

sium (20 cases). In 10 cases, however, the contractions although decreasing from the maximum remained at a level about 10 per cent higher than their original height showing a prolonged beneficial effect. In one experiment where 4 successive injections of potassium chloride were given the rise in the last two cases was followed by a sharp temporary fall in the contractions. This never occurred again although similar conditions frequently prevailed. The increase in contractions with successive injections of potassium chloride showed a tendency to become less but this was not always the case.

The extent of the increase in contraction height varied from 6 to 100 per cent with an average of 39 per cent. Too few experiments were made with the 0.2 per cent potassium chloride-Ringer's and the 0.85 per cent potassium chloride solutions to state definitely whether the increase in contractions varied with an increase in concentration or whether there was an optimum concentration, but the 0.2 per cent potassium chloride-Ringer's solution seemed to be less effective than the stronger concentrations. When the potassium chloride content of the test solution was 0.4 per cent, variations in the concentration of the sodium chloride between 0.9 per cent and 0.58 per cent did not affect the results.

The beneficial effect of potassium was obtained at all stages of the contraction curve, soon after the contractions were begun or later when the muscle was almost completely fatigued and the percentage increase did not show any relation to the height of the contractions before introducing the potassium chloride solution. This marked result of potassium on the contracting muscle was obtained whether the contractions were produced by stimulation of the motor roots, the sciatic or by direct stimulation of the muscle before and after poisoning with curare (fig. 2). It was also not influenced by poisoning of the vasoconstrictor fibers with ergotamine tartrate (Sandoz) and was independent of whether the sympathetic nerves were severed or intact, as would be expected. The improvement in the contractions was not due to an increase in the blood supply due to vasodilatation as the blood pressure usually remained unaltered or increased slightly and the plethysmograph records showed no alteration in leg volume which was not also obtained upon the injection of the Ringer's solution.

For comparison with the above effects of potassium on the contractions of mammalian skeletal muscle a few experiments on the effect of calcium were made in the same manner when the muscle was contracting as a result of stimulating the motor roots. In contrast to the increase in contraction height caused by an excess of potassium in the Ringer's solution, an excess of calcium produced a slight temporary fall in the contraction height. This occurred whenever the concentration of the calcium chloride in the Ringer's solution was 0.49 or 0.65 per cent (figs. 3 and 4),

usually when it was 0.33 per cent and occasionally when it was 0.16 per cent. Concentrations of calcium chloride below this appeared to be without effect. A corresponding reduction in the sodium chloride content was not made in the Ringer's solution when the calcium chloride was increased, because, as noted above, this appeared to be unimportant under the conditions of these experiments. The average decrease in the contraction height was 15 per cent, the fall in contractions began usually 8 seconds after the beginning of the injection and continued for about 10 seconds before the minimum was reached. The recovery was much slower averaging about 36 seconds before the contractions regained their original height. The accompanying blood-pressure tracings showed a slight increase occasionally, indicating vaso-constriction but no apparent changes in the leg volume occurred. The experiments were too few in number to warrant any other conclusions concerning the effects of calcium. They are cited only for comparison with the potassium experiments.

Discussion. These experiments demonstrate that potassium in concentrations exceeding that in blood may have a temporary beneficial effect on contracting skeletal muscle, which is the reverse effect of that generally reported in the literature. This difference may possibly be due to the fact that cats were used in these experiments whereas frog muscles were used in previous experiments. That potassium may have reversible effects, however, even on frog muscle has been demonstrated by Lange (1924) for cell permeability, etc., and by Lapicque and Nattan-Larrier (1926) for muscle chronaxie. Another possible explanation of the effects here obtained may be found in the fact that the potassium solution was considerably diluted with the blood when injected into the circulation. The concentration of the potassium which reached the muscle must have been not over half that in the solution injected but even these concentrations have been reported to depress the excitability of frog muscle. It is possible, however, that the presence of other ions in the blood may alter the action of the potassium ions.

The effect of the potassium is apparently due to a direct action on the muscle itself rather than on the myoneural junction since the effect persists upon direct stimulation of curarized muscle. The explanation of this beneficial effect is impossible with our present lack of knowledge concerning the relation of potassium to skeletal muscle. However, if potassium passes into muscle cells as KOH or in exchange with H ions as suggested by Netter (1928) and Osterhout (1930), this would lead to an increased alkalinity within the cell. Fenn and Cobb (1934), however, found this to be true only to a limited extent.

An excess of calcium, on the contrary, appears to slightly depress the muscle contractions in cats temporarily. This may be due in part to a vasoconstrictor effect although this was not very evident. The reaction

is much slower and less intense in every respect than that produced by the potassium. These results seem to agree with those reported by Benda (1914) rather than with those of Toda (1930), Bouchaert and Belehradek (1927) and Gellhorn (1931, 1932).

CONCLUSIONS

1. The introduction of a small quantity of a solution containing 0.2 to 0.8 per cent potassium chloride into the arterial blood stream of cats produces a beneficial effect on the skeletal muscle as indicated by a marked temporary increase in the height of the contractions. As this result is obtained when the muscle is rhythmically stimulated directly after curarization as well as indirectly through the motor roots, the effect must be on the muscle fibers. This increase in the contractions is not due to an alteration in the blood supply.

2. A similar excess of calcium introduced into the blood stream produces a temporary diminution in the contraction height while solutions with lower concentrations of calcium are without effect.

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THE IMMEDIATE EFFECT OF SPINAL TRANSECTION ON THE CROSSED EXTENSION REFLEX

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Received for publication March 6, 1935

Numerous researches have dealt with the effects of transecting the spinal cord upon the reflexes below the level of transection. The effects have generally been described as depression, and this depression has been commonly designated "spinal shock." In contrast with the commonly described depression is the fact that in the decerebrate animal (cat) the flexion reflex evoked by a single induction shock applied to a sensory nerve, whether recorded mechanically (Sherrington and Sowton, 1915) or electrically (Forbes, Cobb and Cattell, 1923), is greater after transection than before. This fact is interpreted by Sherrington and Sowton (1915) and by McCouch (1924) as due to release of the flexor center from the inhibition incidental to decerebrate rigidity (extensor tonus), the release being effected when the paths from the higher centers involved in maintaining extensor rigidity are interrupted by transection.

Forbes, Cobb and Cattell (1923) found that, whereas the immediate flexor response to the single shock was greater after spinal transection than before, the after-discharge was very much less, so that repeated stimuli evoked discrete twitches instead of cumulative contraction. They also found that, whereas before spinal transection the action currents in the motor nerve in a rapid succession of flexion reflexes (e.g., 4 or 5 a second) showed a marked decline in size from the initial value to a low "fatigue" level, after transection this decline had almost disappeared, successive responses holding up to almost the initial value. Endeavoring to relate these two effects of transection—decreased after-discharge (mechanically revealed) and the much smaller decline in the size of individual electric responses in the motor nerve—they suggested that the decline was due to an increasing "line-busy" effect as the discharge of motor nerve impulses increased in volume, and that the smaller decline after spinal transection was simply an aspect of the decrease in after-discharge following each stimulus, or cumulative discharge during a prolonged series of stimuli.

Later Gerard and Forbes (1928) found that the "line-busy" effect, when directly investigated in the decerebrate cat, proved to be too small and too transient to explain the marked and prolonged decline in the size of motor

nerve action currents in the flexion reflex evoked by a series of single afferent stimuli. A fatigue or "equilibration" effect was invoked to account for the major part of this progressive decline.

McCouch criticized the view that spinal shock, as related to the flexion reflex, could be regarded as a mere decrease in after-discharge, on the ground that the interruption of the cortico-spinal tracts in decerebration introduced the complicating element of extensor rigidity (as mentioned above) with its inhibition of flexor centers, and that, when this effect was removed by spinal transection, the resulting release from inhibition explained the increased size of response. He further emphasized the fact that before spinal transection an interval had elapsed since the initial interruption of the cortico-spinal tracts, which permitted recovery from the real cause of shock.

Forbes and Baird (1929) showed that the increase in the action currents of the flexion reflex, resulting from spinal transection, was essentially the same whether this was performed within half an hour of decerebration or at a later period. This observation did not answer McCouch's main contention that the inhibition of flexors involved in decerebrate rigidity might introduce a confusing factor in the interpretation of the effects of spinal transection on reflexes.

A similar examination of the effect of spinal transection on the crossed extension reflex should have a significant relation to the problems raised by the observations described above, for decerebrate rigidity is a state of sustained extensor excitation, and therefore would not involve an inhibitory effect on the extensor center, such as was invoked to explain the changes in the flexion reflex.

Since the crossed extension reflex is not accessible to the same sort of electrical examination that was used with the flexion reflex (see Forbes and Cattell, 1924), the contraction of an extensor muscle is the index which must be used. This may be supplemented by recording the action currents of the muscle.

METHOD. The essential features of the experiment were the application of single induction shocks or successive shocks at various frequencies to the sciatic nerve in one leg of the decerebrate animal and recording the contractions of an extensor muscle in the opposite hind leg, and rapid transection of the spinal cord without disturbing the set-up of stimulating or recording systems. Cats were used in all experiments.

Under deep ether anesthesia the spinal cord was exposed at the level of the last rib and a thread was passed under it to facilitate transection. It was then packed with moist cotton and the wound was closed. Next the animal was decerebrated at the level of the anterior colliculi. Ether was then withdrawn.

Stimulating electrodes were applied to the left sciatic nerve, severed

distal to the point of stimulation. A right extensor muscle was then prepared for mechanical, and sometimes also for electrical, registration. In nearly all the experiments the knee extensor, vasto-crureus, was used; in a few, the gastrocnemius. In the earlier experiments contraction was recorded isotonically by leading a thread from the lower leg between knee and ankle (or from the foot, when the gastrocnemius was used) to a light lever, partially restrained by an elastic band, recording on a smoked drum. Usually the right sciatic nerve was severed in order to eliminate any possible confusing motions from the lower leg. In the later experiments, isometric recording was used. To this end, the system of drills and lever described by Forbes, Davis and Lambert (1930) was used, the psoas muscles and the saphenous, rectus femoris and obturator nerves being cut, in order to eliminate extraneous motions. With this apparatus, photographic recording is employed by means of the optical system of the string-galvanometer. The galvanometer was then used as a signal to record the time of stimulation, a small part of the stimulating current being led off from the secondary coil into the galvanometer circuit.

For stimulation, a balanced pair of coils was employed and single make and break shocks from it were delivered by a hand-operated mercury contact key designed to insure clean makes and breaks. For repetitive series of stimuli, sometimes the same key was operated by hand in such a manner as to produce from 4 to 8 stimuli per second. In other cases a rotary interrupter was introduced into the primary circuit, providing from 80 to 200 stimuli per second.

In a few experiments the electric response of the extensor muscle was recorded with a string-galvanometer, lead-off electrodes being of the Ag-Ag Cl type connected with the muscle by wicks impregnated with Ringer's solution and agar.

Results. Seventeen experiments were performed, and incidental observations were made on six other preparations in which the spinal cord was transected for the study of other problems. Three of the animals died very soon after spinal transection, becoming moribund rapidly, apparently because of the hemorrhage resulting from the transection. None of these three showed the characteristic picture which was regularly found in almost all those that survived the transection in good condition. The observations made on these moribund preparations are of some interest and will be described later.

In 12 of the 14 preparations which remained in good condition, spinal transection regularly resulted in a change in the crossed extension reflex, similar to that already found in the flexion reflex, but much more pronounced. In the case of the flexion reflex, recorded electrically from the motor nerve, the response to a single stimulus was usually about twice as large after transection as before; in the case of the crossed extension reflex

usually no response to a single stimulus could be detected before transection, whereas after transection a fairly brisk contraction of brief duration was regularly evoked by a single shock. The most striking feature of the

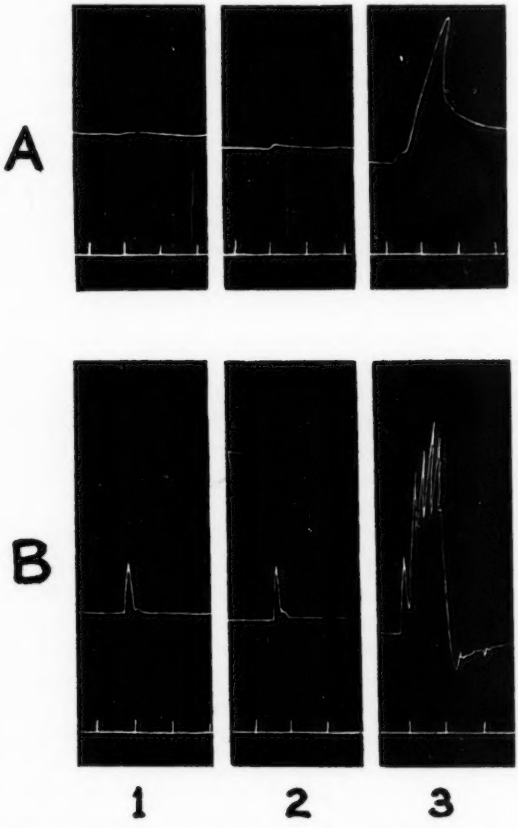

Fig. 1. Contractions of knee extensor, vasto-crureus, in decerebrate cat before and after spinal transection in thoracic region; recorded with approximately isotonic lever. Stimuli applied to opposite sciatic nerve. *A*, 10 minutes before spinal transection; *B*, 4 to 5 minutes after transection; 1 and 2, single break shocks (all the same strength); 3, repetitive make and break shocks by hand-operated key. Time in seconds shown below each record. November 19, 1923.

response after transection was its brevity, for, as in the case of the flexion reflex after transection, it resembled the twitch of a muscle stimulated through its motor nerve. Sometimes an extensor contraction was evoked by a single shock before transection, but in almost every case it was very

small. After transection the single-shock contraction was usually several times as large. Drum tracings showing this change in isotonic contraction are reproduced in figure 1.

The true values of contractile tension, and especially their time relations, are so much better revealed by the isometric lever that the character of the change caused by spinal transection is best shown in the experiments in which this method was used. Figure 2 shows the typical changes resulting from spinal transection, both in the case of single shocks and repetitive series of stimuli.

In contrast with the increased response to a single stimulus after spinal transection, we find a very great decrease in the response to a repetitive series of stimuli. Figure 2 shows this contrast between the effects of spinal transection on the single-shock response and that evoked by a "tetanizing" series, in two experiments. In both cases it will be seen that before transection the single shock evoked little or no contractile response, whereas the series of shocks produced the typical extensor reflex contraction of relatively long latency and gradually increasing contractile tension, indicative of "recruitment" (Liddell and Sherrington, 1923). After transection the single shock evoked a distinct contraction of brief duration, whereas the series of stimuli produced the same sharp initial twitch, followed by weakly sustained contraction at a level never higher (usually lower) than that attained in the initial contraction.

In figure 3 is shown a comparison of the responses to more slowly repeated stimuli before and after transection. These stimuli were delivered by hand, and the interval between them was sufficient to enable the contractile responses to appear as separate peaks after transection, whereas before transection, the stimuli being delivered at about the same frequency, the contraction, starting only after the second stimulus, continued to increase throughout the series. This same difference was also revealed in some of the isotonic records, e.g., figure 1. Examining the separate peaks in figure 3 *B* (after transection), it will be seen that the first contraction is much the largest, and that the size of successive contractions declines rapidly to a smaller value, suggesting fatigue ("equilibration"). Although in most respects the effects of spinal transection are similar in the two reflexes (flexor and extensor) one point of contrast should be noted. After transection the individual responses to the stimuli of a rapidly repeated series show well-sustained magnitude in the flexion reflex, but not in the extensor reflex. But this apparent difference largely disappears when we consider that in the extensor reflex before transection the individual increments of contractile tension, following successive stimuli, are almost lost in the smoothly progressing recruitment of the reflex. After transection the individual responses (fig. 3 *B*) appear as immediate

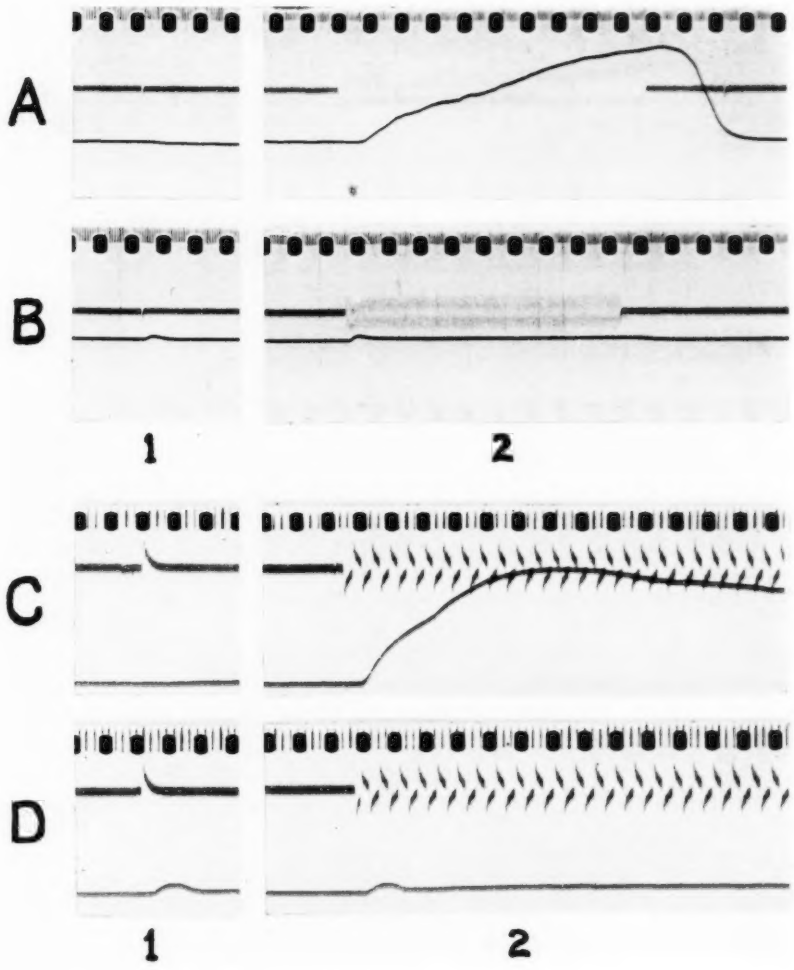

Fig. 2. Crossed extension reflexes recorded with isometric lever before and after spinal transection in two experiments.

In each record, upper line shows application of stimuli (part of induction shocks shunted through galvanometer); lower line shows contraction of vasto-crureus muscle. Time (0.01 sec.) shown above and between perforations at top of each record. *A* and *B*, May 10, 1928; *C* and *D*, December 19, 1934; *A* and *C*, before spinal transection; *B* and *D*, after transection (*B*, 12 min., *D*, 3 min. after).

In each record, 1 is single break shock; 2, tetanizing series of stimuli. Same strength of break shocks in all.

increments of tension as great as or greater than those correlated with the individual stimuli before transection.

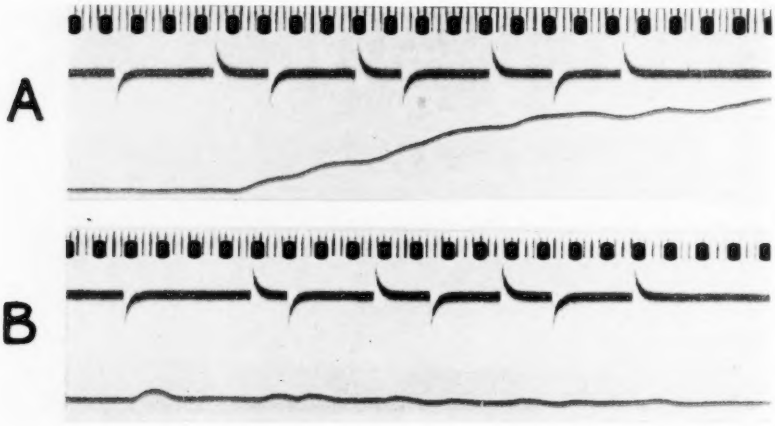

Fig. 3. Comparison of contractile responses in crossed extension reflex to slower series of stimuli, before and after spinal transection. Same arrangement as figure 2. *A*, 3 minutes before transection; *B*, 2½ minutes after transection. December 19, 1934.

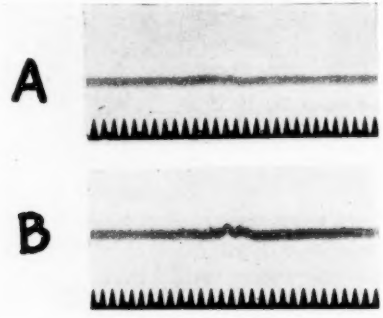

Fig. 4. Electric responses of vasto-crureus muscle in crossed extension reflex evoked by single shocks, before and after spinal transection. *A*, less than 3 minutes before transection; *B*, 10 minutes after transection.

Single break shocks of same strength in both. Time (0.01 sec.) shown below each record. March 30, 1923.

As noted above, electric responses of the extensor muscle were recorded in a few of our experiments. Examples of these are shown in figure 4. They give a comparison of the response to a single stimulus before and after

spinal transection, in the case of the vasto-crureus (knee extensor) muscle. In each case a small initial excursion of the string, caused by the electric artefact, shows the time of stimulation. In each case this is followed after about 30 milliseconds by the electric response of the muscle. In both cases the response seems to consist of two main peaks, suggesting a brief succession of repetitive discharges from the center. Clearly the response after transection is greater than before.

In the three animals which became rapidly moribund after spinal transection, although the flexion reflex persisted, and in one case even showed the characteristic increase, the crossed extension reflex completely disappeared. This suggests that the crossed extension reflex depends on a more vulnerable mechanism than the flexion reflex.

In two other experiments we failed to find the usual increase in the crossed extension reflex evoked by a single shock. In one of these, crossed extension was replaced on transection by crossed flexion, an unusual and anomalous reflex. In the other we found after transection the characteristic shortening of the latency of contraction, but a slight decrease in the size of contraction, instead of the usual increase. This observation was made on the gastrocnemius muscle, which in general showed the characteristic changes less distinctly than did the vasto-crureus. In spite of these exceptional cases, the regularity with which the changes described above appeared in a large majority of our experiments warrants the generalization that they are the usual consequences of spinal transection. This generalization is further supported by the above-mentioned incidental observations in six other preparations, all of which showed an increase in the crossed extension reflex as evoked by a single shock.

Discussion. Our experiments show that in the decerebrate cat spinal transection produces immediate changes in the crossed extension reflex, closely similar to those previously reported in the case of the flexion reflex. In the extension reflex, they are even more marked than in the flexion reflex. These changes may be summarized as increase in the response to the single stimulus and decrease in the after-discharge as revealed in the cumulative contractile effect of a series of stimuli. This cumulative effect, which is the most salient feature of the crossed extension reflex in the decerebrate preparation, is entirely abolished by spinal transection.

As already mentioned, the increase in the flexion reflex upon spinal transection has been interpreted as a release from the inhibition incidental to the sustained excitation of extensor centers which causes the familiar condition of decerebrate rigidity. Clearly this explanation is not applicable to the similar change in the case of the crossed extension reflex, for in this case transection of the spinal cord interrupts a source of excitatory, and not inhibitory, activity coming from the higher centers. This leads to the inference that we are dealing with a general effect of spinal tran-

section upon the lower centers, common to the antagonistic flexor and extensor reflexes, rather than with a specific effect upon the flexor center.

Ruch and Watts (1934), in their comparison of the effects of spinal transection on reflexes above and below the plane of transection, note only a decrease in the extensor reflex in the hind limb. Since they apparently did not look for this reflex in response to single shocks, their results are not in conflict with ours, but rather exemplify the inferences that have resulted from the general use of repetitive stimuli.

We have mentioned above the inadequacy of the "line-busy" explanation to account for the effect of spinal transection on the flexion reflex. In the case of the crossed extension reflex, the increased response to the single stimulus might conceivably be explained as due to a decreased "line-busy" effect from the interruption of the tonic stream of impulses which maintain decerebrate rigidity. This effect might also explain the appearance of distinct twitches in response to a repeated series of stimuli in place of the slow cumulative contraction, but it cannot explain the marked decline in the size of the individual contractions of a series following the first, an effect which appears to depend on something in the nature of fatigue or equilibration.

Our experiments tend to reinforce the idea suggested in previous papers that much of the depression of reflexes called "spinal shock" in the older literature may have been due to the decreased cumulative effect of successive afferent volleys, since repetitive rather than single stimuli were generally used. Matthes and Ruch (1933) have described the crossed extension reflex evoked by a single afferent volley in the chronic spinal cat. They comment that this occurs despite the "depression of extensor reflexes in the spinal condition." Their observation accords with ours, while their comment reflects the prevailing view that depression is general. McCouch (1924) contends that apart from the change from the decerebrate to the spinal condition there is a depression of all reflexes below the level of transection following the first interruption of the cortico-spinal paths, and that, since that effect follows the initial decerebration, the result of a second transection lower down has nothing to do with the depression known as spinal shock. Clearly such an initial depression is not accessible to examination by the method employed in these experiments, since decerebration has already been performed before the observations are begun. Our experiments, therefore, give no answer to the question whether there is or is not such a generalized depression as has been commonly assumed. They do, however, suggest that some of the effects which have been grouped under the term "spinal shock" may have been due to the loss of the cumulative excitatory effect of repetitive afferent volleys, which our experiments show to occur not only in the case of the flexion reflex but even more strikingly in the case of the crossed extension reflex.

SUMMARY

1. Transection of the spinal cord above the lumbar enlargement in the decerebrate cat causes the following immediate changes in the crossed extension reflex: *a*, greatly increased muscular response to a single afferent volley (single induction shock); *b*, abolition of after-discharge and summation of central effects of successive volleys; *c*, decline in the size of individual responses of a series at frequencies which before transection caused cumulative contractile tension.

2. These changes are in the main similar to those previously reported in the case of the flexion reflex, but even more marked.

3. The explanation proposed for the flexion reflex, *viz.*, interruption of inhibitory impulses from higher centers, cannot apply to the extensor reflex, for these centers exert an excitatory effect on the extensor center. The similarity of the effects on these antagonistic centers suggests that the mechanism of the change is fundamentally the same in both.

4. The relation of these observations to theories of "spinal shock" is discussed.

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THE RELATION BETWEEN THE MOTOR AND SECRETORY FUNCTIONS OF THE HUMAN FASTING STOMACH[1]

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Received for publication March 4, 1935

In 1925 Lim, Ivy and McCarthy found that gastric distention provokes secretion in dogs and man. Since tension also gives rise to motility, this led to a consideration of a direct association of the muscular and secretory phases of gastric function. It was accordingly suggested that an enteric reflex secretion might be initiated by the mechanical stimulation of nerve endings as a result of compression. The hypothetical view was expressed that motility is probably invariably related to an increased secretion. Certain unpublished observations of Ivy were cited in confirmation of this supposition, for he had noted that the flow of juice is more abundant during hunger than quiescence. Carlson (1912) however had directly observed that during contraction the secretion of gastric juice was slow, mucin increased, free HCl decreased, and rapidity of secretion was accompanied by motor depression. In 1925 Hoelzel cited the nonuniformity of these views and commented that the abundant juice of Ivy was also higher in acidity. He reported new observations made on man. In an effort to keep intragastric conditions as nearly normal as possible, all but a small sample of the gastric contents were returned after aspiration and the tube was withdrawn, to be reswallowed again except when studies of secretory rate were in progress. The tube was then permitted to remain constantly within the stomach. Motility was subjectively gauged, or studied by the balloon method in the intervals between aspirations. Confirming Ivy, Hoelzel reported that acidity was in general higher during motility than quiescence. However, he found the contents of the fasting stomach smaller during periods of motor activity than quietude and the relative mucin content of the juice increased after strong hunger.

In 1934 we made concurrent inquiries into the secretory and motor phases of gastric function by the method of double intubation which we believe is superior to the one advocated by Hoelzel (Hellebrandt and Dimmitt). Studying the response to a small meal of gruel, we were impressed by the very striking parallelism between these two aspects of the behavior of the

[1] This work was made possible in part by a grant from the Wisconsin Alumni Research Foundation.

stomach. Protracted observations of the spontaneous fluctuations in the acidity of the contents of the fasting stomach (Hellebrandt, 1935) led us to extend our studies to the simultaneous investigation of the secretory and motor conduct of this organ when unstimulated by the presence of food. The results of this inquiry are presented in this paper.

Methods. The fasting subject reported to the laboratory 14 to 20 hours after the last meal. A double or two single tubes were swallowed, one containing the regulation metal olive of the Rehfuss tube, the other, a small balloon of delicate condom rubber. The aspirating tube extended to the lower pole of the stomach. The balloon occupied a position about two inches cephalad. The subject assumed a position of repose in a deck or morris chair and remained asleep or quietly at rest during the period of observation which occupied from three to six consecutive hours. Changes in intragastric pressure were recorded by the use of a tambour and bromoform manometer. Samples of the gastric contents were removed quarter-hourly. In general they were obtained with difficulty during periods of motor activity, and more freely during quiescence. As a rule, only three or four cubic centimeters could be secured and although aspirations were gentle and made with utmost care, occult blood in traces was commonly present in these samples, especially those withdrawn during hunger. Saliva was continuously removed by suction. As in our previous experiments, the observations were made upon healthy young adult women.

Results. In our experience this experiment was difficult to perform, for the double tubes must be perfectly tolerated since nausea and retching vitiate the records. It was at times impossible to obtain samples adequate for titration. Occasionally a reflux of bright yellow bile distorted the acidity curves. During long periods of observation there was a tendency for the aspirating tube to be carried into the duodenum. Most frequently the observations were terminated because of increasingly strong evidence of trauma.

Over a period of 9 months, 10 successful experiments were completed on one subject. These included 20 hunger cycles during 14 of which satisfactory simultaneous acidity curves were gotten. Six additional observations were obtained on two other subjects. One series of aberrant findings was procured from a fourth. We were thoroughly familiar with this subject's ability to secrete hydrochloric acid. Upon one occasion the free and total acidity of her unstimulated stomach reached 79 and 117 respectively. Her response to histamine was normal. She swallowed and held the two tubes without apparent difficulty. Hunger was vigorous and typical. However, repeated aspirations after double intubation yielded consistently small samples which were colorless, thick and glairy. Within an hour they were so viscid that they were poured with difficulty from the tubes in which they were held. Free hydrochloric acid was uniformly absent.

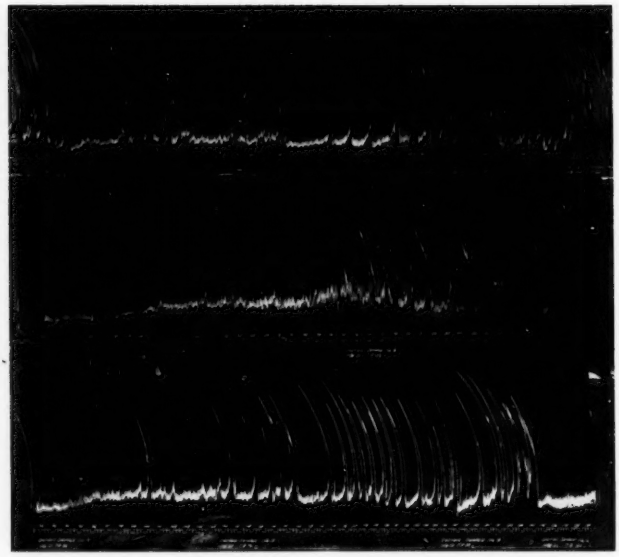

Fig. 1. Kymogram demonstrating the simultaneous secretory and motility data obtained by double intubation of the human fasting stomach. Scratch marks indicate the end of the period of aspiration.

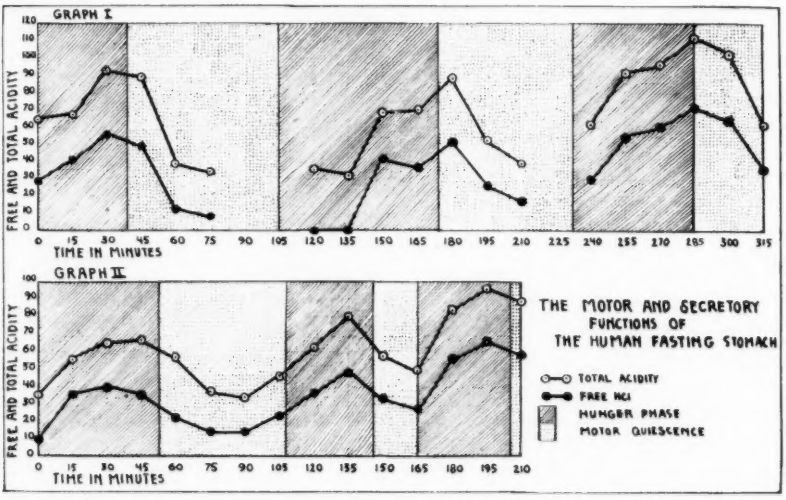

Fig. 2. Diagrams illustrating successive hunger cycles of the human fasting stomach and the fluctuations in the acidity of the gastric contents concurrently aspirated.

Without exception, every other observation showed the following four phenomena: 1, recurrent cycles of motility and quiescence; 2, recurrent fluctuations in acidity; 3, mounting acidity during hunger; 4, a prompt drop in acidity with the onset of quiescence. Figure 1 is a reproduction of a kymogram demonstrating recurrent hunger in subject R. E. B. The periods of relative quiescence are transitory but it is evident that during these intervals, gastric acidity is lower than during the phases of more vigorous motility. Graph I, figure 2, illustrates three successive hunger cycles recorded on subject S. L. H. and the concurrently observed fluctuations in the acidity of the gastric contents. Graph II represents three brief cycles obtained on F. A. H., who never developed the strong hunger and prolonged tetanus of the younger subjects, S. L. H. and R. E. B. However, the juice withdrawn was of relatively high acidity.

COMMENT. We believe that under adequately controlled experimental conditions an invariable relation may be demonstrated between the motor and secretory functions of the human fasting stomach, the acidity of the gastric contents increasing as the motility augments and subsiding with the onset of quiescence. Although normally recurrent cycles of hunger were observed in a subject with achlorhydria, we do not think this throws any light upon the mechanisms involved in the relation noted, because the secretory response was atypical, the excessive production of mucin obscuring the functional response of the acid secreting cells. We have no real evidence suggesting that the uniform relation between acidity and motility is or is not causal. Both phases of gastric function may be under the influence of some extra-gastric factor. We do not know if the two may be dissociated. It seems significant that the parallelism persists whether the stomach is in the fasting condition or under the functionally stimulating influence of food. From the practical viewpoint it seems imperative that cognizance be taken of motility in the evaluation of gastric fractional analyses.

CONCLUSION

Recurrent hunger cycles of the human fasting stomach are associated with fluctuations in the acidity of the gastric contents. These two phases of gastric function augment and subside in unison.

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THE RELATIONSHIP OF THE EXTRINSIC RENAL NERVES TO THE ORIGIN OF EXPERIMENTAL HYPERTENSION

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Received for publication March 8, 1935

It has been suggested that the elevation of blood pressure associated with either inflammatory or vascular disease of the kidneys may be due to nervous impulses originating in the kidneys and conducted by way of the extrinsic renal nerves to the vasomotor centers. This theory is now susceptible of experimental investigation. We have done this by severing the nerves of the renal pedicles before subjecting animals to two different procedures for the production of hypertension of renal origin.

Methods resulting in hypertension in dogs. The first method employed is that of Goldblatt, Lynch, Hanzal and Summerville (1934), an ingenious procedure for constricting the renal arteries.[1] A small silver screw clamp is placed on the main renal arteries and tightened sufficiently to impede slightly the blood flow.

The usual lumbar incision was made to expose the kidney. It was freed with its vessels from perirenal fat and peritoneum, the renal capsule being left intact. When denervation of the vessels was desired, they were cleaned of all visible nerves as far as the aorta and vena cava. In practice, it was found better to sever them close to the aorta where they are gathered in compact bundles and to strip them as they fan out toward the periphery. Nerves leading to the ureter were avoided. In some of the experiments the pedicle was then painted with phenol (3 per cent) dissolved in alcohol. A silver clamp was placed around the main artery and the screw tightened to compress the artery gently. Constricting the vessel more tightly results in thrombosis. The kidney was carefully replaced; the vessels must not be twisted. The wound was closed with gut reinforced by interrupted silk sutures. In some experiments clamps were applied to both renal arteries, and in others to the remaining artery after one kidney was removed.

In a second method, both kidneys were transplanted to a position directly under the skin of the flank, and the wounds allowed to heal. They

[1] Ligation of branches of the renal artery was performed by Pässler and Heineke (1905), Carrel (1909), Cash (1924), Mark and Giesendörfer (1930), to reduce renal tissue by necrosis of a portion of it. The method of Goldblatt, Lynch, Hanzal and Summerville produces renal ischemia instead.

were then treated with x-rays. If the remainder of the body is protected with lead plates the explanted kidneys can be irradiated without the use of deep x-rays. Treatment consisted of two erythema doses to each kidney at approximately four to six-week intervals. The details of this method, and the results of chemical studies of the blood, will shortly be reported in another communication.

Measurement of blood pressure. The van Leersum carotid loop method was found to be the most satisfactory procedure for the measurement of blood pressure in dogs. In this country the method has been described and modified by Cohn and Levy (1920) and more recently by Goldblatt[2] and his associates. It consists in sewing a length of the common carotid artery within a flap of skin so that it lies free on the neck of the animal.

Certain precautions have been found useful. If the skin is thin and loose the carotid loops are less likely to break. The skin flaps should be loose, since the tissues become edematous during the first few days after operation. Otherwise the sutures may burst or thrombi may form. Large pieces of absorbent cotton held in place by a gauze bandage have been found the most useful dressing, because they cling to the wound and prevent dirt from entering under the edges of the bandage, and because it is sufficiently soft to avoid constriction of the carotid loop. The dressing should be changed on the second or third post-operative day; afterward the wound should be treated with ointment such as butesin picrate until it is thoroughly healed.

Blood pressure measurements were made daily under environmental conditions as nearly similar as possible. The dogs were easily trained to sit quietly while the blood pressure was being measured. From ten to twenty readings were taken, and *that one accepted* which *seemed to represent* the most stable blood pressure level. The differences in pressure were not greater than 30 mm. of mercury.

Usually the operation on the carotid artery and unilateral nephrectomy were performed under amytal anesthesia (60 mgm. kilo given intraperitoneally). After complete healing had occurred (15–25 days), and the level of the blood pressure had been established (20–30 days), the renal nerves were severed and the clamp applied to the other renal artery.

Chemical methods employed in investigation of the blood. The hemoglobin was measured by the method of Van Slyke and Neill (1924), urea by the gasometric urease method (Van Slyke, 1927) plasma protein by the method of Howe (1921), and lipids by the gasometric method of Kirk, Page and Van Slyke (1934). Examinations of the blood were made after fasting for 24 hours. Water was, however, given freely.

[2] We are extremely grateful to Dr. Harry Goldblatt for many helpful suggestions concerning the application of their method, and for the silver clamps. He was also kind enough to allow us to undertake the problem, although he had had it on his program of future work.

RESULTS. In five dogs, about six weeks after performing unilateral nephrectomy, the remaining kidney was denervated and a clamp applied to the renal artery. Measurements showed that unilateral nephrectomy did not cause a significant rise in the level of the blood pressure, but constricting the renal artery did so shortly after recovery from anesthesia. A few days after operation the pressure in some cases measured 220 to 290 mm. Hg (figs. 1 and 2). If the renal nerves were left intact, a similar response occurred in two control animals. Denervation alone did not cause a significant elevation in blood pressure in two animals. Elevation of the blood pressure has already persisted for 240 days in animals apparently in good health. A trace of albumin was occasionally found in the

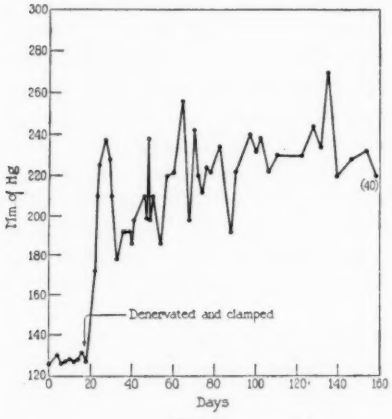

Fig. 1

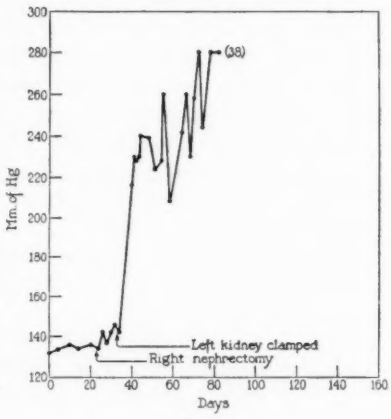

Fig. 2

Fig. 1. Dog. No. 40. Rise in arterial blood pressure following denervation and clamping of the renal pedicle.

Fig. 2. Dog. No. 38. Rise in arterial blood pressure following denervation and clamping the left kidney, after preliminary removal of the right kidney.

urine, as well as a few hyaline and granular casts. Hematuria was observed rarely, and there was little, if any, loss in power to concentrate urine. The arterioles in the fundus of the eyes appear to be constricted, but no hemorrhages or exudates have appeared. When both renal arteries are clamped, a rise in blood pressure similar to that just described has been observed.

No significant change in the chemical constituents of the blood has been detected, though a small but definite increase has occurred in the concentration of hemoglobin (table 1). Urea was either normal in amount or slightly increased, except when too great constriction of the renal artery caused renal insufficiency. Renal insufficiency, measured in terms of the urea content of the blood, was not a necessary nor usual accompaniment of arterial hypertension. The proteins in plasma were normal.

Lipids in the blood, including total fat, total and free cholesterol, phosphatide phosphorus, lipid amino nitrogen, and total lipid nitrogen, all were found to be normal in amount. This result also corresponds with clinical observations in the absence of signs of the nephrotic syndrome (Page, Kirk and Van Slyke, 1935). The only phenomena which may be unusual concern the ratio of free and ester cholesterol to total cholesterol, for free cholesterol increased at the expense of the esters during the course of the exper-

TABLE 1

Blood constituents of dogs with hypertension

NO.	HEMOGLOBIN IN BLOOD IN TERMS OF O_2 CAPACITY	UREA NITROGEN	PLASMA PROTEINS	PLASMA LIPIDS MGM. PER 100 CC.									AVERAGE BLOOD PRESSURE
				Total lipid carbon	Total lipid	Cholesterol			Lipid amino nitrogen	Total lipid nitrogen	Total lipid phosphorus	Total phosphatides	
						Total	Free	Ester					
Unilateral nephrectomy. Remaining kidney denervated and clamped													
	vol. per cent	*mgm./ 100 cc.*	*mgm./ 100 cc.*										*mm. Hg*
Dog 38:													
Control	19.0	10.60	6.89	540	702	170.0	62.0	108.0	5.8	24.2	18.0	423	128
5 days after clamping	17.2	10.37	7.06	445	578	138.5	48.8	89.7	3.0	17.6			230
19 days after clamping	16.6	27.96	7.06	617	802	157.5	64.5	93.0	5.7	23.1	15.7	369	228
37 days after clamping	24.48	79.35	6.00	677	880	183.8	93.3	90.5	8.6	32.8	16.7	382	>280
One kidney denervated and clamped													
Dog 40:													
86 days after clamping	23.13	18.58	6.75	547	711	72.9	48.8	24.1	6.9	45.5	13.2	310	238
126 days after clamping	22.40	13.87	5.49										
Unilateral nephrectomy. Remaining kidney denervated and clamped													
Dog 43:													
Control	18.66	13.6	7.25	663	861	194	69.8	124.2	5.8	38.1	18.2	428	136
12 days after clamping	16.41	24.8	7.63	988	1284	184	111.3	72.7	6.7	35.9	22.9	538	218
51 days after clamping	21.98	19.97	6.79	596	774					14.4	13.3	312	>280
91 days after clamping	22.71	19.56	7.37	743	976	86	66.6	19.6	6.5	36.2	21.2	498	>280

Clinical observations. Dog 38. Animal died three days after the last blood examination. Post-mortem examination showed that a portion of the kidney was severely damaged.

Dog 43. This animal was quite sick following clamping of the renal artery. It is probable that the slightly elevated urea nitrogen and total fat were due to this.

iment. The liver may have been damaged, but no convincing proof has been found that this is so.

The results of the second method, irradiation of the transplanted kidneys, parallel those of the first. Bilateral renal denervation was performed on six animals, and six were employed for control purposes. Rises in blood pressure occurred in both groups after exposure to five to seven erythema doses of x-ray to each kidney. The level of the blood pressure usually

was not greater than 200 mm. of Hg, and was much more variable than that observed when the renal artery was clamped.

SUMMARY. Two methods have been employed in dogs, which have resulted in renal hypertension. The first, that of Goldblatt, Lynch, Hanzal and Summerville, consisted in constriction of the renal arteries by means of a silver clamp. The second consisted in transplantation of the kidneys to a position under the skin of the back, and, after healing of the wound had occurred, in irradiation with x-rays.

The first method produced sharp and sustained arterial hypertension regardless of whether the kidneys had been denervated. Except for rise in the concentration of hemoglobin in the blood, the constituents, including urea nitrogen, plasma protein, total fat, total cholesterol, lipid amino nitrogen, total lipid nitrogen and phosphatide (calculated from the lipid phosphorus) were not significantly altered. The amount of free cholesterol was increased somewhat at the expense of cholesterol ester. Increased blood urea concentration did not, as a rule, accompany the hypertension. Goldblatt and his associates (1934) have shown, by means of the urea clearance test, which is a more delicate measure of renal function, that hypertension and renal efficiency are independent of each other in dogs when clamps are applied to the renal arteries.

The results of the second method are parallel to those of the first, in that hypertension occurred as readily in animals in which bilateral renal denervation had been performed as in those which were the subjects of transplantation and irradiation alone. Hypertension was of shorter duration and not so severe as that produced by clamping the renal artery.

CONCLUSIONS

1. Since the production of arterial hypertension in dogs by constricting the renal arteries, or by irradiation of the kidneys with x-ray, is not affected by preliminary stripping of the renal pedicle of its extrinsic nerve supply, these nerves do not appear to participate in the genesis of renal hypertension.

2. Hypertension produced by constriction of the renal arteries does not result in significant changes in the proteins or lipids of the plasma. The hemoglobin content of the blood is slightly elevated. Renal efficiency, as measured by the content of urea in the blood, is not markedly altered, and bears no relationship to the height of the blood pressure, as Goldblatt, Lynch, Hanzal and Summerville have already found.

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AUGMENTATION OF THE PHYSIOLOGIC RESPONSE TO INSULIN

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Received for publication February 26, 1935

In spite of the voluminous research on insulin, successful attempts to slow down the resorption from parenteral depots to correspond more nearly to a physiologic rate of liberation have not been forthcoming.

Various protein precipitants and metallic salts have been found by Maxwell (1934) to markedly retard the rate of resorption of hypophyseal gonadotropic extracts. Since the physical and chemical characteristics (unpublished data) of the protein aggregate associated with the hypophyseal gonadotropic principle in some respects resembles that of the insulin complex, it appeared possible that compounds which were effective for the gonadotropic principle might also prove effective in retarding the resorption of insulin in the tissues. This paper deals chiefly with the action of basic ferric salts in prolonging insulin hypoglycemia.

METHODS. Adult rabbits and rats were used as test animals. The animals of each experiment were divided into two groups and fasted for twenty-four hours previous to dosage. One group of rats was used as controls and was given two units of insulin (Lilly) per kilogram of bodyweight intramuscularly. The other group was dosed simultaneously with two units of the same insulin preparation to which had been added basic ferric chloride. After one week of rest the control and the insulin plus ferric chloride groups were reversed and the experiment repeated. This procedure controls any individual variation or change in insulin tolerance. The rabbits were subjected to a similar procedure with the exception of receiving one unit of insulin per kilogram body weight. Blood sugar values for the rats were determined by the Folin micro-method, and for the rabbits by the Schaffer-Hartmann method.

Insulin (Lilly) U 20, was added to a dilute aqueous solution of ferric chloride which had been previously neutralized to the point of precipitation. The pH of the solution of insulin after the addition of the basic ferric chloride ranged between 4.5 and 6. The mixture was diluted to contain two rabbit units of insulin and four milligrams of ferric chloride per cubic centimeter. The control animals were dosed with a similar dilution of insulin alone.

RESULTS. The mean average of the blood sugar values at various time intervals for the rats is shown in figure 1. It will be noted that the values for the control and the iron groups are identical at one and a half hours. At the third hour the mean of 34 ±3 for the iron group (17 rats) is significantly less than the value of 46±3 for the control group (17 rats). At the fifth hour the most striking effect is noted. The value for the controls at this time has risen to 69 ±3.7, while that for the basic iron group has remained between 30 and 35 ±3.4. At the eighth hour the blood sugar values of the controls have approached normal. At this time there was

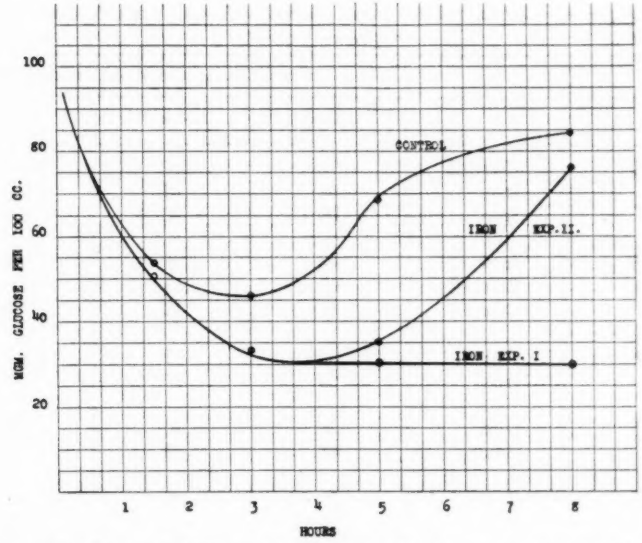

Fig. 1. The effect of addition of basic ferric chloride to insulin upon the blood sugar response of rats.

a difference in the response of the animals in the two experiments which comprise the iron group. In experiment I (6 rats) all the animals in the basic ferric chloride group were prostrated at the eighth hour, while in experiment II (11 rats) only one animal showed marked symptoms of hypoglycemia. This difference in response is illustrated by a division of the curve (fig. 1).

One group of three rats which had received the same dose of insulin to which had been added 4 mgm. of tannic acid per cubic centimeter previous to injection gave a blood sugar curve identical with that of the group receiving insulin plus basic ferric chloride. These results are not tabulated and

are mentioned only as evidence that the effects noted are due simply to a retardation in the rate of insulin resorption in the tissues.

The blood sugar data for the rabbits receiving insulin plus basic ferric chloride are essentially the same as for the rats. The data for the rabbit experiments are presented in table 1 in the order in which the experiments were performed.

Sahyun and Blatherwick (1928) in this laboratory found that after a pronounced hypoglycemia had been produced in rabbits, a ten-fold increase

TABLE 1

The effect of addition of basic ferric chloride to insulin upon the blood sugar response of rabbits

RABBIT	TREATMENT	BLOOD SUGAR VALUES IN MGM. PER CENT		
		3 hours	5 hours	8 hours
G	Control	76	130	106
	Iron	64	64	95
	Iron	50	40	45
W	Iron	62	58	92
	Control	62	112	116
	Control	30	92	103
7	Control	32	82	106
	Iron	24	20*	
6	Iron	56	96	106
	Control	64	98	
X	Control	56	88	105
	Iron	50	50	45
B	Iron	42	64	88
	Control	70	100	100

* Insulin convulsion.

in the dosage of insulin produced little change in the rate of fall of the blood sugar level during the first hour and a half. The effect noted was the prolongation of the hypoglycemia. Likewise the effect upon the blood sugar level produced by the addition of ferric chloride to insulin is not evident at one and a half hours, and is just significant at three hours after administration. It will be noted however that at the fifth hour the blood sugar of the group receiving insulin alone again approaches normal, while that of the group receiving insulin plus ferric chloride has not yet commenced to rise and only approaches the normal value at the eighth hour,

or in approximately half the cases even later. Sahyun and Blatherwick found that in order to produce differences in the blood sugar level of rabbits corresponding to those obtained by us at the fifth hour, it was necessary to increase the insulin dosage four- to ten-fold. This analogy indicates that when insulin is resorbed from the tissues at a rate corresponding to the more uniform physiologic liberation, the activity is greatly increased. Whether the decreased activity observed when insulin is rapidly liberated in the tissues is due to inactivation, or to an adrenalin defense mechanism, awaits investigation.

SUMMARY

The addition of basic ferric chloride to insulin augments the physiologic response as measured by blood sugar changes in both rabbits and rats.

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SEX-DRIVE IN RATS[1]

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Received for publication January 22, 1935

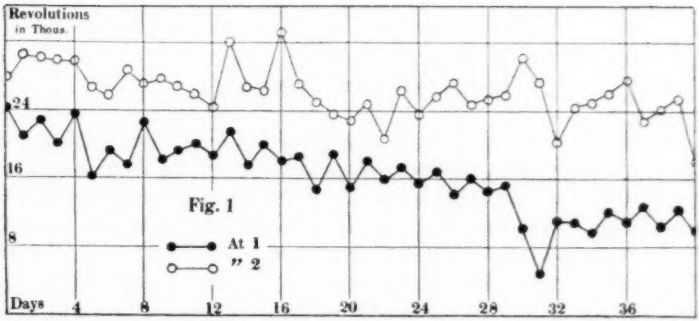
In measuring the efficiency of rats by placing them in revolving cages one is confronted with the problem of the effect of adjacent rats on the animal under experimentation. If an effect on the activity of the experimental animal is produced by adjacent rats then additional variable factors would be introduced which might influence or nullify the results of the investigation. The object of this paper is to present certain observations which show that adjacent rats do exert an influence on spontaneous activity and must be considered in dealing with problems of efficiency.

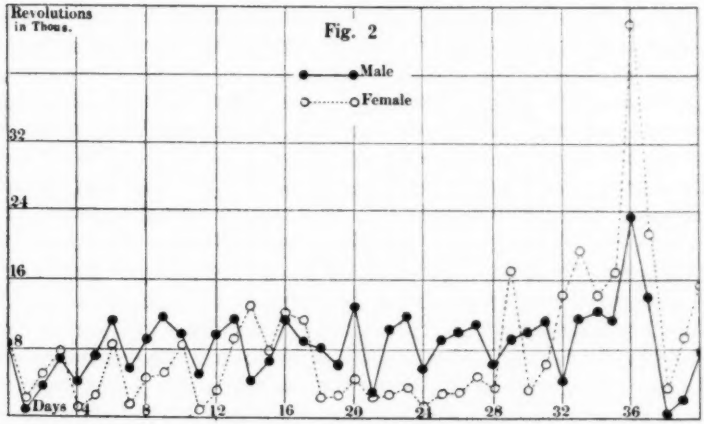
It is well known that female rats during sexual life show marked rhythmic peaks of activity which are synchronous with the oestrual period and that the isolated males show almost a uniform curve of activity—wholly devoid of rhythm (see fig. 1). We found, however, that the activity curves of male rats in cages near a female tended to follow the rhythm of the female activity. We concluded that there may have been at least two factors involved in modifying the usual male activity curve: 1, stimulation due to oestruation, known as *sex drive*; 2, increased activity of the female may have excited the rhythmicity in the male.

In order to throw some light on these possibilities males were subjected to three different laboratory conditions.

1. Eight males were isolated in a room with their cages arranged in a single row. The circumferences of these cages were 30 cm. apart. These were designated *At 1* to *At 8* consecutively. The activity curves for 40 days of the first two (*At 1* and *At 2*) in the row are shown in figure 1. During this period the ages were from 163 to 203 days. *At 1* showed a tendency toward a two-day rhythm. The greatest daily range of 3700 revolutions occurred between the fourth and fifth days. This range is much less than that usually shown by females (see fig. 2). The average daily run for the whole period was 8210 revolutions. The average daily run for the next

[1] This research has been assisted by The Department of Physiology, the National Research Council through the Committee for Research in Problems of Sex, the National Live Stock and Meat Board, Standard Brands, Inc., Albers Bros. Milling Company, Corn Products Sales Co., Swift & Company and the Golden State Company, Ltd.





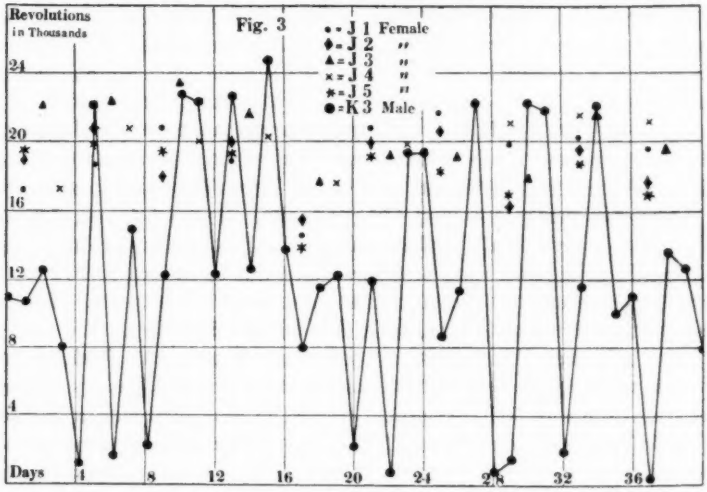


Fig. 1. Activity curves of males, *At 1* and *At 2*, which were adjacent to each other, but not near a female.

Fig. 2. Activity curves of male (solid lines) and female (dotted lines) of one of the groups of three, arranged in the order of male, female, male. The male curve represents the average run of the two males.

Fig. 3. Showing the activity curve of one male, *K3*, and the oestrual peaks of closely adjacent females *J1*, *J2*, *J3*, *J4* and *J5*.

rat, *At 2*, for the same period was 13036 with a maximum daily range of 3800. During this same period the third rat (*At 3*) in the bank of cages averaged 10229 revolutions daily. The average daily run for the eight males in this group was 4723 revolutions. Figure 1 shows that *At 2* showed no tendency to a two-day, or any other rhythm. It also exhibits only a few cases of synchronous peaks of activity in the two curves. This number was approximately 15 per cent of the total possibilities. From this we conclude that increased activity of one male rat had little effect toward stimulating an increased activity on the part of adjacent males, the circumferences of whose cages were 30 cm. apart.

2. Three groups of three rats each were segregated in the colony room about three meters from any other animals. These groups were designated L, M and N. The order of the arrangement of the three rats in each group was identical. A female occupied the middle cage and a male on each side. The cages of each group were 15 cm. apart at their circumferences. Since each cage was 45 cm. in diameter the nearest either of the males could approach the female was approximately 30 cm. An increased activity of the males, synchronous with that of the oestrual peaks of the female, would indicate that the female not only exerted a sex drive but that it could exert its influence over a distance of at least 30 cm. The activity curves of the two sexes of one group (M) for the same forty-day period are shown in figure 2. During this period the males were from 268 to 308 days of age and the female from 309 to 349 days. For the sake of simplicity and clearness the male curve represents the average daily run of the two males. By consulting this figure it is readily seen that the male curve approaches very closely that of a typical female curve and that the oestrual peaks of the female are approximately in synchronous arrangement with the high peaks in the male curve. It is further seen that during the greatly reduced activity of the female from the 18th to the 29th day the mean activity of the male is not only maintained, but the peaks still respond chronologically to the slight oestrual peaks of the female. The lack of complete accordance of the peaks in the two sexes may have been due to the sex drive stimulus having occurred earlier or later than the indicated female peak. We have no suggestion for the discrepancy shown on the 29th day. We thus see that not only was the activity of the male thrown into fairly definite rhythmic arrangement but that the daily fluctuations very greatly increased from a maximum of 3800 in figure 1 (rat At 2) to a maximum of approximately 14000 revolutions (rats M1 and M3). The average daily number of revolutions for the female during this period was 8078; for the males it was 8614. The average daily activity of these two males was 3891 revolutions greater than the daily average of the eight males in the former group. Since the rats of the former group were 100 days younger than M1 and M3, and were in the naturally more active phase of life, this difference in

average daily activity is more significant. This became more marked when the activity of these two groups at the same ages was compared. The average daily run then was 4691 greater for M1 and M2 than that of group 1. From these data we infer that the sex-drive stimulus was the main factor in bringing about these results and that the mere activity of the female had little or no effect. We thus see that the close proximity of a female induced both rhythmicity and greater activity of the adjacent males.

3. Five females, ages 139 to 179, and five males, ages 490 to 530, were used in the third group. One male, K2, died early in this period and its record was not used. The disposition of the animals in the third arrangement was as follows: A bank of cages containing the five females, J1 to J5, and a bank of cages containing the five males, K1 to K5, were placed with the cages end to end as close as their supports would permit. This brought the ends of the cages 9.5 cm. apart. K3 male could therefore approach that close to J3 female, but would be separated from J2 and J4 females by approximately 45 cm. and from J1 and J5 by 100 cm. All of these females had regular four-day periods except J4 which showed a six-day interval between the 23rd and 29th days. Three other cages containing males (K6, K7 and K8) of the same ages as the males K1 to K5 were placed at some distance from any female but in the same room. Fortunately the females came in heat on three successive days in the order of J1, J2 and J5 on the same day followed by J3 and J4. Then followed a day during which all were sexually passive. We thus had groups of three-day periods of sex-drive stimulus separated by a day of no stimulus. The first and third days this stimulus was exerted over a distance of from 45 to 100 cm., while on the second day only over 9.5 cm. Figure 3 illustrates the effect of these individual stimuli on the single male K3. For the sake of clearness only the oestrual peaks of each of the females are indicated. They not only show the chronological order, but also indicate the number of revolutions each female ran that particular day. In the second arrangement previously described the sex-drive stimulus was present only one day in four while in this case it was operating three successive days in four. This figure shows that the approximation of a four-day rhythm exhibited by the males in figure 2 was completely eliminated in K3 and that the response of this male was due to one or more of the three possible stimuli in each four-day period. It is further noted that the peaks of activity and the daily fluctuations were greatly increased and that the days of great activity were usually followed by those of little activity. This suggests a state of lassitude or almost complete exhaustion during which no response was given to the stimulus. The maximum daily range was almost 22,000 revolutions. K3 was the most active of the four males. The average daily run of this male was 12209 revolutions and of the five females 10863

revolutions. The average daily run of all the males in this group was 4651 revolutions. The average daily revolutions of the three remote males, K6, K7 and K8, during this same period was 250. Since the age of this male during this period was from 490 to 530 days, an age during which the activity of males is relatively greatly reduced, a direct comparison of the activity curves in figures 1 and 2 does not present an accurate picture of the effect of sex drive on this animal (K3). The average daily runs of the males of figures 1 and 2 during the same ages as K3 were 3144 and 8240 respectively. A comparative summary of the activity of the different groups at the ages of the three periods used in figures 1, 2, and 3 is given in table 1. These averages show the effect of an increasing sex-drive stimulus on the activity of an adjacent male rat. In the later records it was further noted that between the ages of 550 and 650 days the response by marked

TABLE 1

Average daily revolutions of the groups at the ages given for figures 1, 2, and 3

AGE	GROUP At 1 TO At 8				GROUP M			GROUP J AND K					GROUP K (REMOTE FROM FEMALES)	
					Males		Female	Females (J)		Males (K)				
	No.	Av.	At 1	At 2	No.	Av.	Av.	No.	Av.	No.	Av.	K 3	No.	Av.
163 to 203	8	4723	8210	13036		*		5	14010	5	12591	14715	3	3198
268 to 308	8	4012	276	156	2	8614	8078	5	8478	5	6492	9633	3	508
490 to 530	8	3144	266	768	2	8240	9793	5	3988	4	4651	12209	3	250

* Not in revolving cages.

peaks of activity by the males became very sporadic and occurred more often as single responses which were separated by intervals of several days' duration. Occasionally groups of two or even three successive oestrual-like peaks were elicited. With increasing age of the males the characteristic responses due to sex-drive stimulus finally ceased even though the adjacent females were still showing their regular oestrual peaks of activity.

Figure 3 shows that, with only a few exceptions, the response of the male coincided with an oestruation. A few instances will be cited. On the second day it reacted to female J3 and on the third day to J4. On the fourth day there was no sex-drive stimulus and the male showed little activity. On the fifth day J1, J2, and J5 were in heat and a burst of activity equivalent to almost 20 miles was elicited. We cannot state what part, if any, the oestruation of J1 and of J5 had in this response. Females placed at different distances from males would determine the maximum

distance over which the sex-drive stimulus could operate. After this excessive run the male appeared to be so fatigued that it did not respond the following day to the closest female, J3. The seventh day it reacted to J4. On the 9th, 10th and 11th days a response was to all three of the possibilities. Obviously the vitality of the male did not permit a vigorous response to all of the stimuli. We attribute this lack of complete agreement of the high points of activity of the male to that of an oestrual peak either to complete fatigue, or to oestrus having occurred a few hours before or after the reading of the revolution recorders. A later oestruation of J3 would account for the male curve on the 27th, 31st, and 35th days. A few hours before or later would cause the high point of the male curve to appear twenty-four hours before or after the oestrual peak. Hourly readings would be necessary to get an accurate representation of the results.

From the above we are led to conclude that when male rats are confined in revolving cages which are placed as close as 45 cm. from females in the prime of life, the spontaneous activity of the males will be modified and increased by a sex-drive stimulus. We have not determined over how much greater a distance this stimulus will cause a response. Our records show that these stimuli do not operate on the same sex. It follows, therefore, that in experiments on male rats, which involve spontaneous activity, the possible sex-drive stimulus of adjacent females must be taken into consideration.

SUMMARY

When male rats were confined in revolving cages to ascertain spontaneous activity the following results were observed:

1. When two cages, each containing a male rat, were closely adjacent, the increased or decreased activity of one did not seem to influence the activity of the other.

2. When a male and female were placed in separate cages so that the animals approached as near as 45 cm., the activity of the male was not only greatly increased, but was also changed into a marked rhythm similar to and approximately synchronous with that of the female oestrual rhythm. The stimulus which caused this is known as sex drive.

3. With the addition of a greater number of females in close proximity to the male, his activity was still further increased and was no longer manifested in the characteristic oestrual-like peaks elicited by the presence of a single female if oestruation occurred on different days.

4. With increasing age of the male beyond 550 to 650 days the effect on the male of the sex-drive stimulus became less and less and finally ceased.

5. The activity or sex-drive stimulus of one female did not seem to affect the activity of an adjacent female.

THE PRESSURE CHANGES INDUCED IN THE VASCULAR SYSTEM AS THE RESULT OF COMPRESSION OF A LIMB, AND THEIR EFFECT ON THE INDIRECT MEASUREMENT OF LATERAL PRESSURES

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Received for publication January 16, 1935

It has already been shown that though accurate estimates of end pressures may be made by the Riva Rocci method, particularly if the "formo-ozsillatorisch" criteria of von Recklinghausen are used, yet during the process of compression and decompression considerable changes in directly recorded pressures may be observed, not only locally (Bazett and Laplace, 1933a, fig. 4), but also in the opposite femoral artery (loc. cit. fig. 3). In view of the fact that the criteria adopted as indications of lateral pressures (Bazett and Laplace, 1933b) were not definitely established as valid, further work to compare direct and indirectly measured pressures was undertaken, and this has shown that some of the pressure changes previously attributed by us to reflex adjustments have in reality a simpler mechanical causation. In this paper, consequently, data will be presented to demonstrate the local changes in pressure that are produced by compression, and the effect of these on the accuracy of criteria for measurement of lateral pressures will also be discussed.

The previous work of Erlanger and Hooker (1904), of Merke and Müller (1925), and of von Recklinghausen (1930) as well as the data reported by ourselves (1933a) makes it quite certain that the differences between end and lateral systolic pressures are considerable (10 to 33 mm.); consequently, in measurement of pulse pressure by the Riva Rocci method, the relationship of any criterion used to one or other of these pressures should be determined, since the whole principle of the Riva Rocci method for systolic pressure involves obstruction of the stream. Considerable differences of opinion exist as to the magnitude of the error involved. Maltby and Wiggers (1932) as the result of compression of a vessel in a clamp concluded that "the experimental evidence does not favor the view that systolic pressure readings obtained by clinically applicable criteria are necessarily too high"; on the other hand, Frank and Wezler (1931) considered that the reflection of waves may cause errors of 30 per cent or more in estimations of pulse pressure.

The problem, therefore, was to determine the effects produced on the actual pressures by the process of compression, and what criteria, if any, indicated the pressures existent in the system before interference by compression. This problem was attacked from three standpoints: *a*, experiments were conducted with a circulation schema essentially of the type described by Erlanger and Hooker (1904); in this the segment compressed was varied in type and size and the actual pressures were estimated by two optical manometers (Wiggers' pattern) inserted in the system on either side of the compressed segment; optical oscillograms of the pulsations of the compressed segment were also obtained; b, experiments similar to those with the schema were conducted with the compression chamber inserted in the thigh of a dog, so that the pulsatile changes were generated by the heart and retained their normal pulse form; c, the criteria demonstrable by oscillatory methods were compared in man with those obtained by auscultation on the same or opposite arm.

METHODS. a. *Experiments with a schema.* The schema is shown in figure 1; it resembles that described by Bazett (1924). The segment compressed in the glass compression chamber consisted of tubing of varying size and thickness, or of a section of dog's carotid of somewhat smaller bore than the smallest rubber tubing. The optical oscillometer was of the type described previously (Bazett and Laplace, 1933a). Corrections were made for any incomplete pressure equilibrium indicated by a shift in the oscillometer zero. The changes in the tube during compression were also watched through the glass and critical changes were signalled.

b. *Experiments on animals.* Dogs were used and were anesthetised as described in the previous paper (Bazett and Laplace, 1933a).

A chamber of the same type as that used in the schema was inserted in the thigh, with the manometers arranged as in the schema experiment. The femoral artery was dissected from the groin to the knee, and its lateral branches were tied. A section some 6 cm. in length was removed and was inserted into the chamber, and the upper end of the whole compression system was tied into the central end of the femoral artery. The lower end of the excised artery in the chamber was connected to the lower end of the main artery by a glass cannula, short length of heavy rubber tubing and the manometer. Heparin (20 mgm. per kilo) was injected intravenously to prevent clotting. In such experiments good oscillometric records could be obtained but the local circulation was abnormal since most of the branches of the femoral were tied, and the normal velocities in the vessel must have been considerably reduced.

In other animal experiments the procedure was consequently modified. A single Wiggers manometer with a simple cannula was inserted into the profunda femoris in such a way that its orifice was at right angles to the stream in the femoral artery. The peripheral pulse was recorded from a

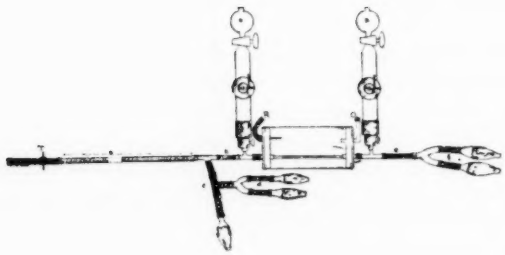


Fig. 1 Circulation Scheme

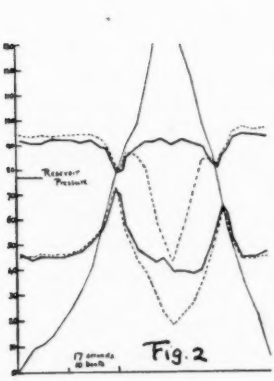


Fig. 2

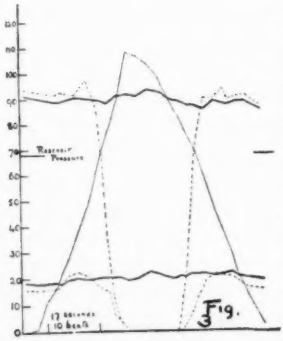


Fig. 3

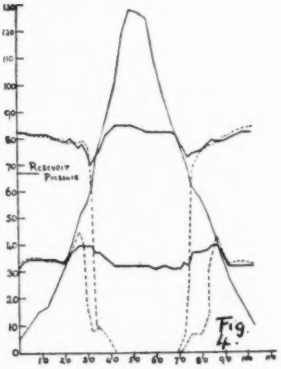


Fig. 4

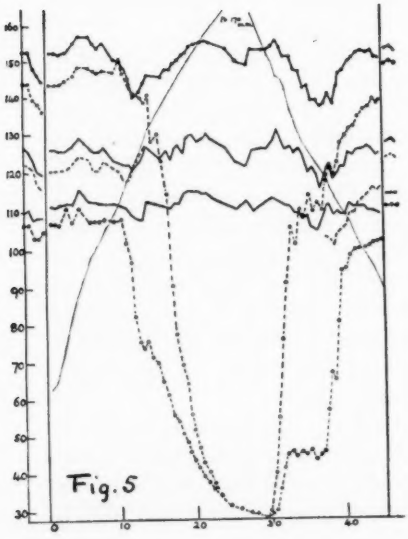


Fig. 5

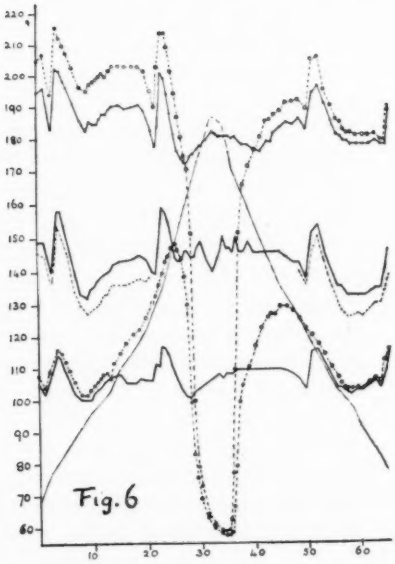


Fig. 6

cannula inserted into a peripheral vessel in the leg; it indicated the presence or absence of a peripheral pulse but not its exact form. This method could only be used to investigate the accuracy of systolic estimates, since space did not allow the insertion of an adequate oscillometric compression system.

Standardization in the animal experiments was carried out in the usual way and corrections were made for the saline levels in the manometers and also for variations in the position of the limb from the horizontal.

c. In experiments on man the triple bag system with optical recording was used on one arm, and the pulse below was also recorded; by a T-tube connection an ordinary armlet on the other arm was inflated at the same pressure, and the various stages of auscultatory sounds were signalled.

Fig. 1. Schema: water entered from a reservoir through a mechanically operated valve *T*; light rubber tubing of 8.3 mm. bore with 0.03 mm. wall and 27.5 cm. in length was used for section *a*; heavy walled tubing of 6 mm. and 2 mm. wall for all other connections with lengths—*b*—3.5 cm., *c*—1.0 and 3.5 cm., *d*—3.5 and 3.0 cm., *e*—4.5 cm., *f*—3.0 and 4.0 cm. Tube *O* connected the compression chamber with the oscillometer, tube *R* with an air reservoir, the outer chamber of the oscillometer, the manometer and the supply of compressed air. The outflow tubes were packed with cotton, and controlled by screw clips.

Fig. 2. Effect of compression on pressures in schema when compressed segment consisted of tubing 8.3 mm. bore, 0.03 mm. wall, of length 8 cm. The thin continuous line indicates compression pressure, heavy continuous lines—systolic and diastolic pressures recorded by the manometer on the proximal side, dotted lines—systolic and diastolic pressures recorded by the manometer on the distal side of the compression. The abscissae indicate time, the ordinates—pressure in millimeters of mercury.

Fig. 3. The effect of an experiment similar to those of figures 2 and 4, except that an excised carotid artery was used for the compressed segment, and the whole system was perfused with normal saline. Symbols as for figures 2 and 4.

Fig. 4. The effect of an experiment similar to that shown in figure 2, except that the compressed segment consisted of tubing of 4 mm. bore with wall of 0.008 mm. thickness and with an outer supporting cover of thin silk. Symbols are used as in figure 2. The outflow from tubes *c* and *d* was high as compared with that from tubes *f*. Abscissae indicate time in seconds.

Fig. 5. The pressures recorded in a dog with a section of femoral artery removed and reinserted within a compression chamber. The thin continuous line indicates the compression pressure, the heavy continuous lines the directly recorded systolic, dicrotic and diastolic pressures on the proximal side of the compression; the dotted lines the systolic, dicrotic and diastolic pressures on the distal side of the compression. At either end of the figures are indicated the values observed in a series of pulse cycles obtained before and after the application of the compression pressure. The open and closed circles indicate the cycles measured, since all the cycles are not recorded. Part of the actual record is reproduced in figure 8. Abscissae indicate time in seconds, ordinates—pressures in millimeters of mercury. Corrections for gravity have been applied to the values obtained in the distal manometer, as explained in the text.

Fig. 6. The pressures obtained after the production of aortic regurgitation in the experiment illustrated in figure 5. The symbols used are the same as for figure 5. Part of the record is reproduced in figure 9.

In other experiments a single receiving tambour at the elbow below the triple bag was connected not only to a recording Frank capsule but also to a stethoscope, so that both sets of observations were made on the same arm. The results obtained by both methods were similar.

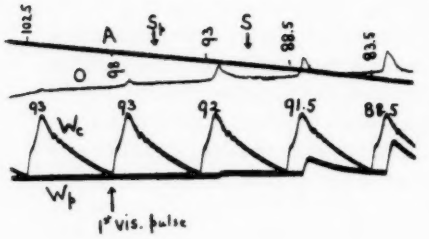
RESULTS. *Pressure changes induced by compression. In the schema:* The results obtained are illustrated in figures 2 to 4 and 7. Systolic pressure rose considerably above the static reservoir pressure of some 70 mm., even though the velocities of fluid movement were not high (mean velocity of systolic inflow about 20 cm. a second). During compression there was a marked fall of systolic and rise of diastolic pressure at compression pressures above the diastolic level, and the effect was the greater the larger the volume of the compressed segment. When the compression pressure exceeded the systolic level, this effect changed to one in which the systolic pressure was somewhat raised. There was no evidence of flow along the angles of the compressed segment except with the thicker tubing (fig. 2), where separation of the two sections was never complete. When the compression separated the two sections of the schema for some part of the cycle, the pressure changes proceeded quite independently in the two sections, and the rise in diastolic pressure might be more in evidence in the peripheral section. This difference between the two sections could be modified by altering the rate of outflow in the tubes; the increase was greater in the peripheral section than in the central when the rate of outflow centrally (c and d) was much more rapid than peripherally (f). A recordable peripheral pulse persisted until the compression pressure exceeded the initial lateral systolic pressure by some 3 or 4 mm., though it was obliterated at a pressure several millimeters below the maximum "end" systolic pressure. On visual examination, when the compression pressure exceeded this maximum systolic pressure, a slight bulging of the upper conical end of the compressed segment with each pulse beat could be observed; as the pressure was allowed to fall below this systolic level but when it was still above the lateral systolic level before compression, the wave was able to pass the compressed segment as a small "bolus" of streamline shape which only partially filled the compressed segment. Such pulsations represented small volumes which were inadequate to give a recordable pulse below the compression, but they were indicated by a quite definite increase in the oscillometric pulsation. Part of an actual record obtained with a segment of a dog's carotid artery within the compression chamber, reproduced in figure 7, shows the development of an increased oscillometric change as the "bolus" type of wave was transmitted, the later appearance of a recordable peripheral pulse (both of which changes may develop at compression pressures above the true lateral systolic pressure) and the production of a peripheral pulse pressure which at moderate compression considerably exceeded the central.

In dogs: The essential changes are illustrated in figures 5 and 6, which show the pressures recorded in a dog with a glass chamber inserted in the thigh; figure 5 shows the results in the normal dog, figure 6 those obtained later after the production of a moderate aortic regurgitation by the method previously described (Bazett, 1924). Parts of the actual records are shown in figures 8 and 9. The differences between the two sets of data depended on the aortic lesion, since intermediate changes were obtained in the period between these records with a smaller aortic leak. The peripheral end of the thigh was somewhat higher than the central, and the values of the peripheral manometer have been corrected for this gravity effect; the actual values were 4 mm. lower than those charted.

Though complicated by rhythmical physiological variations the figures demonstrate the presence in the animal of factors similar to those acting in the schema. In both there was a lowering of systolic pressure during compression, a peripheral pulse was recordable even when the compression pressure exceeded the pressure recorded by the central manometer, and the "bolus" type of wave transmission continued even when the compression pressure exceeded that indicated by the central manometer by 21 mm. (figs. 5 and 8). Neither before nor after aortic regurgitation was there any definite increase in the centrally recorded systolic pressure at the highest compression pressures, but the transmission of the pulse waves was never entirely prevented. Reflected waves produced only minor variations of the systolic and dicrotic waves, though at pressures just below the systolic level a marked negative reflection, creating an apparent deep incisura of peripheral origin, was a conspicuous feature of the centrally recorded pressures, and this wave appeared to contribute to the formation of a negative wave in the oscillogram.

Neither before nor after aortic regurgitation was the central diastolic pressure much affected by compression. In the normal animal the peripheral diastolic pressure fell rapidly and earlier than the systolic, so that the peripheral pulse pressure was increased; but after aortic regurgitation the peripheral diastolic pressure was raised enormously, and the peripheral pulse pressure reduced.

A marked slowing of the pulse wave transmission, such as has been described by others, was always seen, and often a sharp negative wave preceding the main upstroke of the peripheral pulse. Such waves may be clearly seen in the original of figure 9 at compression pressures above the diastolic level, and are also just detectable in figure 8 at pressures between the diastolic level and 140 mm., except that during deflation at the diastolic level these waves are masked by a negative wave of much longer duration and of quite different origin. This longer wave also often occurred in the schema and by the use of single pulses could be demonstrated to belong to the end of diastole, and not, like the other, to the beginning of systole.



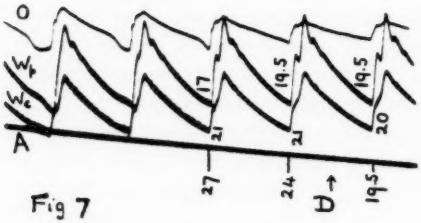
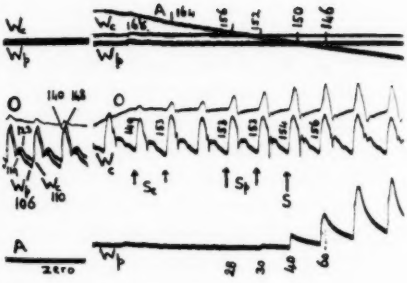


Fig 7



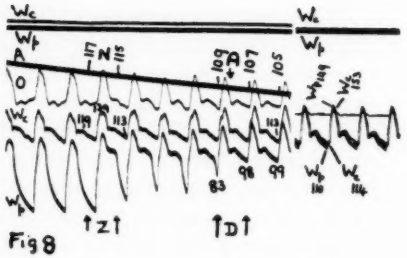


Fig 8

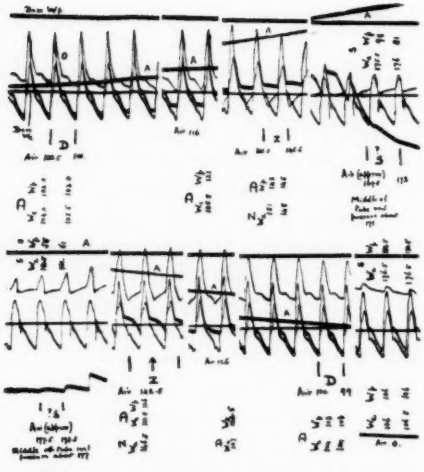


Fig 9.

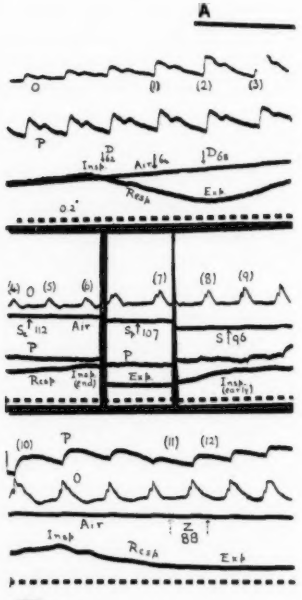


Fig 10.

Riva Rocci criteria. Diastolic pressure: The essential criteria were changes in the character of the oscillogram, and in the peripheral pulse, and the development of an initial negative wave in the latter under favourable conditions. The changes in the oscillogram may be seen in figure 7 which shows a record obtained in the schema and, therefore, uncomplicated by physiological variations. In the earlier pulses of the lower half of the figure the flattened area at the end of the cycle, dependent on the presence of a flat empty tube, is a conspicuous feature of the oscillogram; at the third pulse this disappears, and the more rapid rate of fall that precedes it only survives. Under conditions when the compressed segment was partially collapsed at the end of a cycle, the following pulse produced a much more rapid volume change at its commencement and, consequently, a much more rapid upstroke of the oscillogram; this change disappears only at the last pulse of figure 7; the air pressure of 24 mm., therefore, exceeded the true pressure and that of 19.5 was below it, as indeed was the case.

Fig. 7. Part of an actual record obtained in the schema with compression of a segment of carotid artery within the chamber. *A* indicates air pressure, *O* the oscillogram, W_c the Wiggers manometer central to the compression, W_p that peripheral to the compression; *Sp*, *S* and *D* indicate the position of indirect estimates of systolic and diastolic pressures by the criteria discussed. The values indicate the pressures in millimeters of mercury of the air pressure, and in the upper section of systolic pressure in the near manometer, in the lower section of the diastolic pressures in the two manometers. The appearance of the first visible pulse of the "bolus" type is also indicated.

Fig. 8. Part of the actual record of the experiment plotted in figure 5. W_c indicates the arbitrary base line and record of the centrally placed Wiggers manometer, W_p the base line and record of the peripheral manometer; *A* indicates the air pressure, and its zero is shown in the first section obtained before compression; *O* is the oscillogram. The numbers indicate the values of pressures at various points. The marks *Se*, *Sp*, *S*, *Z* and *D* indicate the points at which the various criteria for systolic, dicrotic, *Z*, and diastolic pressure were considered to occur. Visible pulsations of the "bolus" type appeared to pass the compressed segment, even at pressures above 166, but they were extremely small. The apparent development of the peripheral pulse before that of the central is due to parallax.

Fig. 9. Part of the record of the experiment plotted in figure 6. The oscillogram and part of the air pressure record has been slightly retouched to assist reproduction. The figures given are inserted above or below the records with a letter to indicate the phase of the cycle measured. Remarks as to the probable pressure at the center of the compressed segment represent estimates based on corrections for gravity and an assumption of the loss of 2 mm. pressure through friction.

Fig. 10. Part of record of air pressure, peripheral pulse, oscillogram, and respiration on compression and decompression of the arm in man obtained with more rapidly moving paper than that usually employed to illustrate the shape of the waves. The air pressure and respiratory records are so marked, *O* is the oscillogram, and *P* the peripheral pulse below the cuff. The small pulse (11) is seen to depend on the lowered systolic pressure level of early expiration.

The situation is somewhat more complicated in the dog. Thus in figure 8 it may be observed that at a compression pressure of 109 mm. the oscillogram showed the increased rate of fall, after demonstrating the flat area in the two previous pulses; the peripheral pulse showed no true negative wave of short period but did show an abnormally abrupt fall of pressure at the end of diastole. This wave of diastolic origin probably depended on the relation of outflow to elasticity constants in the peripheral section, when this section was separated from the rest of the system by the closed and empty compressed segment. Both these criteria disappeared at a pressure of 107, and the diastolic pressure was estimated as 108 mm. The pressure recorded by the central manometer was 113 but since there was, in the absence of compression, a fall of pressure of 8 mm. from gravity and friction along the compressed segment, there was no evidence of any error. The presence at this time of a very low diastolic pressure in the distal segment did not interfere with the accuracy attained.

After the production of aortic regurgitation (fig. 9) a compression pressure which somewhat exceeded the diastolic pressure instead of producing in the oscillogram a flattened terminal area at the end of diastole produced, on the contrary, only an increased rate of fall, and the delay in the production of the flattened area seemed to vary with, and be dependent on, the increase observed in the peripheral diastolic pressure. At the true diastolic level there was a very definite and abrupt development of an initial negative wave of short duration immediately preceding the upstroke of the peripheral pulse, as well as a less definite increased abruptness of the oscillographic upstroke. These criteria were uninfluenced by the peripheral increase of diastolic pressure; they may be seen more clearly in records obtained on man with a somewhat increased rate of paper movement (fig. 10). The occurrence of the rapid upstroke of the oscillogram is seen in pulse 1, the initial negative wave is just detectable in the peripheral pulse in pulse 2, and in the oscillogram in pulse 3. An initial slow upstroke at the start of the oscillogram may also be noted; its origin may be in the upper cone of the compressed segment. The diastolic pressure can be estimated as between 62 and 64 mm. on the basis of the rapid oscillographic upstroke preceded by a slurred start, and as between 64 and 68 mm. on the basis of the development of a negative wave.

The schema and animal experiments do not permit any decision as to which is the more accurate criterion, for in neither type of experiment is there present within the compressed area a series of vessels of different size with presumably somewhat different internal pressures. Normally in man the two criteria occur at pressure levels which are almost identical (see table 3) but recent unpublished observations (Bazett, Scott, Maxfield and Blithe) indicate that under certain conditions they may develop at defi-

nitely different pressure levels, and the increase in steepness of the oscillometric curve may be rather gradual. Mainly on theoretical grounds the diastolic pressure has, consequently, been estimated in man by first noting the lowest compression pressure at which a peripheral negative wave is developed (either in the oscillometer or peripheral pulse) and then determining the nearest pressure below this at which the oscillometer attains its maximum steepness of upstroke. Diastolic pressure is taken as the mean between this compression pressure, and that at the next pulse.

The accuracy of the estimates in a series of experiments in the schema and on animals, where these complications are absent, is indicated in tables 1 and 2. In table 1 selected data from schema experiments are given, which demonstrate that the speed of inflation or deflation (nos. 1–3) has no great effect on the accuracy of the mean of the compression and decompression estimates. In table 2 data obtained in experiments on dogs are given without distinction between compression and decompression; in this table the values included under "range" and "mean" represent the direct estimates made before and after compression of the limb; those labelled "simultaneous" indicate the range of directly measured pressures at the time the indirect estimates were made; the indirect estimates are the mean values of all estimates made during compression and decompression. The average error in the estimate of diastolic pressure was in the schema experiments ± 2 mm., and in the animal experiments ± 1 mm. The estimate was in the schema usually about 1 mm. too high, in the animal experiments 1 mm. too low.

The estimation of *dicrotic pressure* could not be made in the schema. In dogs it was indicated in the oscillograms by a dicrotic wave which arose from the descending limb of the main pulsation, and which grew in size as the air pressure was lowered (fig. 8); at pressures slightly below the dicrotic pressure, a dicrotic wave also became evident in the distal pulse. Both in animal experiments and on man there may be developed, during compression, a secondary wave which resembles a dicrotic wave; such a wave may be seen in figure 10 in the oscillograms of the first two waves of the lowest section (pulse 10). The most marked and earlier of the waves in pulse 10 is certainly not a dicrotic wave, but an effect of the compression system; the less marked later wave on the down-stroke may or may not be a dicrotic wave; its fading out as the pressure falls and absence in pulse 11 does not exclude it being a dicrotic wave, since there were marked respiratory variations in pressure in this experiment. Probably this latter wave represents a true dicrotic wave, and figure 10 illustrates the difficulties that may be encountered in determining the presence or absence of a dicrotic wave when there are marked respiratory variations in pressure. When the dicrotic notch is very marked it is probable that the compressing pressure may be below that of the dicrotic wave even when this wave

TABLE 1

Indirect measurements of lateral pressure in the schema

NO.	COMP. TUBE	COMP. OR DECOMP.	UNOBSTRUCTED PRESS	SIMULTANEOUS COMPARISONS								REMARKS
				Systolic						Diastolic		
				Direct	Se	Direct	Sp	Direct	∞	Direct	Est.	
1	Rubber	Comp.	81/32	83	90	78 83	86	74 78	80	32	34	Rapid comp. and decomp. Mean ests: Se. 87.5. Sp. 82.5. S. 75.5. D. 31.5
		Decomp.	81/32	84	85	84 78	79	78	71	32 31	29	
2	Rubber	Comp.	82/34	85	83	76 78	81	73 76	75	34 35	33	Slower comp. and decomp. Mean ests: Se. 85. Sp. 80.5 S. 73. D. 31.5 (See fig. 4)
		Decomp.	82/32	85	87	80 78	80	76 73	71	35 32	30	
3	Rubber	Comp.	81/35	84	83	76 80	76	76	74	37	38	Still slower comp. and decomp. Mean ests: Se. 82.5. Sp. 76. S. 72. D. 35
		Decomp.	81/35	84	82	82 81	76	77 74	70	37 36	32	
4	Rubber	Comp.	90/33	94	90	87 90	84	87	83	35 38	36	Slow comp. and decomp. Mean ests: Se. 90.5. Sp. 84. S. 81. D. 36
		Decomp.	90/33	94 92	91	87	84	87 85	79	38 33	36	
5	Rubber	Comp.	87/4	100	105	97 99	94	94	88			Very slow comp. and decomp. Very open outflow. Mean ests: Se. 101.5. Sp. 88.5. S. 87.5
		Decomp.	87/4	100	98	93 90	83	93 92	87	4	8	
6	Rubber	Comp. and decomp.	103/16			108 107 107 108 107 105	101 105 98	106 107 107 108 108 107	103 105 102			Slow fluctuations of air pressure at systolic level. Very open outflow. Mean ests: Sp. 101. S. 103
7	Artery	Comp.	89/18			90 91	85	90 91	85	18	19	Rather rapid compression. Mean ests: Se. 93. Sp. 87. S. 87. D. 19.5
		Decomp.		94	93	90	89	90	89	21 20	20	
8	Artery	Comp.	90/20			92 94	(100)	92 94	(100)	11 12	(16)	Rapid compression and these values doubtful. Mean ests: Se. 98. Sp. 96. S. 91. D. 22
		Decomp.		94	98	93 92	96	92 91	91	21 20	22	
9	Artery	Comp.	93/13			92	94	91 92	85	12 17	15	Mean ests: Se. 99. Sp. 93.5. S. 89. D. 17.5
		Decomp.		93	99	93	93	93	93	18 17	20	

rises from the base line of the oscillogram, and that a duplication of its peak (Bazett and Laplace, 1933b) should, in this case, be taken as the criterion. This condition has not, however, been duplicated in animal experiments.

The mean discrepancy of the estimates from the mean value of the dicrotic wave before compression was in the experiments listed in table 2 ±4 mm., with the average estimate some 2 mm. too high.

The estimation of *end systolic pressure*, from the occurrence of a sudden increase in the size, and change in character, of the oscillometric curve,

TABLE 2

NO.	SYSTOLIC			DICROTIC		DIASTOLIC		REMARKS
	Direct Est.	Indirect Est.		Direct Est.	Indirect Est.	Direct Est.	Indirect Est.	
		Sp.	S.					
1. Range	102–115			91– 97		80–86		Artery in chamber branches tied
Mean	109			95		84		
Simultaneous	101–110	107	100	90– 97	97	80–86	83	
2. (a) Range	136–152			115–126		103–112		As in 1
Mean	145			121		108		
Simultaneous	149–153	156	151	115–120	119	107–109	109	
(b) Range	149–158			120–126		107–111		As in 2(a). (See fig. 8)
Mean	153			123		109		
Simultaneous	142–153	153	149	117–119	121	107–110	107	
(c) Range	156–164			107–111		92– 98		As in 2(a) and 2(b) but with moderate aortic regurgitation
Mean	159			109		95		
Simultaneous	151–161	153	149	110–116	114	96– 97	94	
(d) Range	173–188			130–144		99–104		As in 2(a) to 2(c) but with greater degree of regurgitation (See fig. 9)
Mean	180			137		102		
Simultaneous	174–180		174	144–149	143	102	100	
3. (a) Range	145–176							Artery intact. Branches not tied. Big resp. variations made comparisons difficult
Mean	159							
Simultaneous	153–182	163						
(b) Range	142–196							As in 3(a) but with aortic regurgitation
Mean	180							
Simultaneous	190	185						
4. (a) Range	159–193							As in 3(a). Irregular fluctuations in blood pressure
Mean	?							
Simultaneous	158–173	160	160					
(b) Range	149–160							As in 4(a) but with aortic regurgitation
Mean	155							
Simultaneous	152–157	154	151					

can be made accurately, as was previously reported by Bazett and Laplace (1932a); in the schema the indirect estimates averaged 1 mm. too high, and the mean variation from the direct estimate was ±3 mm. The estimation of *lateral unobstructed systolic pressure* was difficult owing to the complexities introduced by the changes in the real pressure as the result of the compression. At compression pressures somewhat below those

giving the end systolic pressure criterion a recordable peripheral pulse was developed, a criterion designed Sp in the tables; this criterion might be developed at compression pressures either above or below the true lateral systolic pressure according to the relative values of the varying sources of error. Thus in the schema experiments (table 1) the mean discrepancy was ±3 mm., and the mean of the estimates was 1 mm. too low, while in the dog experiments (table 2) the mean discrepancy was ±4 mm., and the mean of the estimates was 2 mm. too high. The criterion previously suggested by us for lateral systolic pressure, namely, the presence of a shoulder on the oscillogram or on the peripherally recorded pulse gave values which were too low both in the schema and in the dog; thus in the schema (table 1) these estimates had a mean value 3.7 mm. too low, and

TABLE 3

Comparison of auscultatory and optical estimates

SUBJ.	SYSTOLIC				DIASTOLIC					
	Opt. Sp.	Ausculț. syst.	Discrepancy		Opt. neg. wave	Opt. oscill.	Ausculț. fading.	Ausculț. disapp.	Discrepancy*	
			Mean	Extreme					Mean	Extreme
1	115.0	115.0	0	0	58.5	57.3	55.0	35.5	±4.0	+3
										−17
2	118.0	116.0	−2	0	71.0	67.8	67.5	47.8	±7.0	+11
				−8						−21
3	113.0	113.5	+0.5	+2	61.0	58.2	55.8	40.5	±6.2	+8
				0						−14
4	126.7	126.2	−0.5	0	78.7	77.0	82.0	73.7	±8.3	+18
				−4						−10
5	139.7	136.4	−3.3	0	69.3	67.3	59.3	40.5	±10.7	+9
				−9						−23

* Discrepancy estimated between optical oscillometric and auscultatory fading.

nearly all the discrepancies were negative, while in the dog experiments the mean value was 5.3 mm. too low and again nearly all the discrepancies were negative.

Comparison with auscultation criteria. The auscultatory criterion for systolic pressure checked well with that of the first recordable peripheral pulse (Sp). There was rarely a discrepancy of more than a single cycle, which, with the slow pressure changes used, only involved differences of 2 or 3 mm. The "lateral systolic" criterion (S) occurred at pressure levels where the sounds became much louder or assumed a murmur character. With slow compression or decompression the sounds changed gradually and showed respiratory variations, and the observer had considerable difficulty in analysing the changes, so that exact comparison was difficult.

Table 3 gives values for systolic pressure obtained on 5 subjects by auscultation and by the appearance of a recordable pulse below the cuff (Sp criterion). The method used was that where observations were made on the same arm. The practical identity of the two estimates is obvious, and the optical record is shown to be slightly the more reliable. All the subjects were normal, except subject 5, who had aortic regurgitation.

Comparisons of the diastolic criteria were also difficult with slow pressure changes. Sounds continued, especially during decompression, at compression pressures below the diastolic pressure indicated either by the peripheral negative wave or the character of the oscillometric curve. These sounds were sometimes marked, particularly in aortic regurgitation, and caused confusion in judging the auscultatory criterion. The estimation of diastolic pressure from the fading of sounds was in fair agreement with estimates from the oscillometric curve (see table 3); in fact, if subject 5 with aortic regurgitation be excluded, the mean estimates by the two methods were almost identical. However, the optical method appeared to be the more reliable. Though some of the variations in any single subject were undoubtedly due to physiological variations, of respiratory or other origin, yet some were certainly due to experimental error; the mean deviation from the means for the different individuals, excluding the subject with aortic regurgitation, was ± 3.9 mm. for the negative wave criterion which was usually very sharp, $\pm$ 4.8 mm. for the oscillometric change which was less definite, and was ± 6.2 mm. for the auscultatory criterion. The two methods did not agree in the subject with aortic regurgitation, where the auscultatory estimate was definitely much lower. The mean deviations from the mean were in this subject (12 observations) ± 3.4 for the negative wave criterion, ± 4.6 for the oscillometric change, and ± 7.7 for the auscultatory criterion. The consistency of the optical methods was the same as, or somewhat better than, that observed in normal subjects, that of the auscultatory method was definitely less. The two optical criteria agreed well with one another in this series; the change in the oscillogram gave almost invariably values some 0 to 4 mm. lower than those indicated by the negative wave.

Discussion. The complexities introduced into a pulsating elastic system filled with fluid when a segment is compressed appear to depend on several factors; of these one, long recognised, namely, the development of either positive or negative reflected waves appears to play a minor rôle except at the systolic level; a second, which, though obvious, seems to have attracted little attention, is the fact that when the segment is closed the two parts of the elastic system are completely separated, and the pressure changes in each part develop separately according to their own conditions such as rate of outflow and volume elasticity coefficient; a third factor is a decrease in the volume elasticity coefficient of the system as a

whole, as the distensibility of the compressed segment becomes determined by the air column rather than by the vessel wall; this factor has long been recognised in relation to its effect on the pulse wave velocity, but its effect on the pressures developed has received little attention; a fourth factor is the alteration of the wave form by the development of "breakers" as the result of the great contrasts in the speed of transmission of different parts of the pulse wave.

Reflected waves. While a negative reflection of the pulse wave is a conspicuous feature at compression pressures just below the systolic level when the peripheral vessels are empty, positive reflections of the same wave do not develop rapidly at higher compression pressures nor are they large. The air system which leaves an open cone at the upper end of the compressed segment appears to act as a cushion which prevents any sudden reflection of the wave, particularly when the compression pressure does not much exceed the systolic level. Consequently, the differences between end and lateral pressures recorded by the central manometer were not as great as those observed when the artery was closed by a clip (Bazett and Laplace, 1933a). Though the minimizing of this effect may have been partly due to the cannula holding open the upper cone, and to a diminution of the velocity head by the ligature of branches, the differences between end and lateral systolic pressures were still subnormal, even when these factors were excluded by the use of a somewhat different system (table 2), so that at least part of the minimising effect may be considered as a concomitant of all Riva Rocci systems.

The independence of pressure changes in the separated sections and general effects on distensibility. These two factors are closely related and may be considered together. In systole the system reacts as a whole, and if the compressed segment is open sufficiently long the systolic pressures of both sections are the same; the effect of the air column on the volume elasticity coefficient, however, damps the systolic wave and lowers systolic pressure. In the diastolic phase the two sections are separated by the compressed segment, and the pressure changes develop independently in the two sections according to their own rates of outflow and volume elasticity coefficients. If the compressed segment is closed early in diastole, the duration of diastole is thereby prolonged in the distal segment, and diastolic pressure consequently tends to fall to a lower level, so that the peripheral pulse pressure is usually greater than the central. Since the volume elasticity of the compressed segment is the lowest of the whole system, at one stage in the cycle it tends to empty its contents rapidly and to supply for a while the loss of the system as a whole, while maintaining a temporary plateau in the pressure curves. According to the conditions in the system this discharge of the contents may occur mainly into the central vessels or mainly into the peripheral, and this factor is concerned in the effect of vary-

ing the rates of outflow centrally and peripherally on the relative changes in central and peripheral diastolic pressure in the schema. The effect produced on diastolic pressure varies with the volume of the compressed segment, but a volume, which may be quite ineffective if discharged into a large central system, may produce considerable changes, if directed into the smaller peripheral section. The data that have been given are all explicable on this hypothesis.

In the experiment of figure 5, the slight upward slope of the limb would tend to cause closure of the compressed segment first at its peripheral end, and the consequent central discharge of the contents of the single artery would not alter pressures in the large central reservoir appreciably; on the other hand, after production of aortic regurgitation the rapid central fall of pressure would tend to cause closure at the central end and discharge of the contents in the opposite direction peripherally, where the contained volume would be by no means negligible. The marked increase in the peripheral diastolic pressure of figures 6 and 9 would depend then on two factors: *a*, this peripheral section was filled in systole at the high systolic pressure which resulted from aortic regurgitation, but during diastole it was completely separated by the compressed segment from any diastolic effect of aortic regurgitation; and *b*, this section received the contents of the compressed segment as was indicated by the maintenance of the peripheral diastolic pressure at a plateau which had the same pressure (after subtracting the 4 mm. gravity correction) as the air system, while the oscillometric curve demonstrated that the compressed section at this time was discharging and had not emptied. In contrast, figure 8 shows evidence of only a very small and brief peripheral discharge of the contents. This effect of the discharge of the contents may be recognised in most of the graphs in that it tends to raise the diastolic pressure parallel with the change in the compressing pressure, as must be the case since the latter supplies the expelling force.

These factors must be quite important even in the indirect estimation of pressures in an intact animal, for the pressure changes reported previously in the opposite femoral of a dog during the compression of a thigh (Bazett and Laplace, 1933a; fig. 3) are also most readily explained on the same hypothesis. The fall in systolic and rise in diastolic pressure observed during compression in the normal condition should be expected, since the volume contained in the compressed thigh would be considerable, and in the normal sloping position of the thigh the contents would be discharged centrally, and should be effective in altering central diastolic pressure; on the other hand, after aortic regurgitation the systolic effect should be preserved, but the contents of the compressed thigh should be directed peripherally and be quite ineffective in altering central diastolic pressure. The data of both experiments, however, imply that in the thigh the pres-

sure fall at the central end of the femoral artery with aortic regurgitation is much more rapid than at the peripheral end of the same artery, and that there is a considerable retrograde flow in diastole, even though in the experiment here reported there was only a hole about 6 mm. in diameter in a single aortic cusp.

Alterations in wave form. The formation of secondary waves, which precede the main pulse and which typically include a sharp negative deflection, has been ascribed by Bramwell (1925) to the formation of breakers as the result of the very different rates of transmission of different parts of the wave. This hypothesis is supported by the observations of Frank and Wezler (1931) that the rate of transmission of the main wave is, at compression pressures above the diastolic level, less than 1 meter a second. According to Bramwell's hypothesis, these secondary waves should develop more readily the steeper the upstroke of the pulse and the greater the differences between the transmission rates of the different parts of the pulse curve. In favour of Bramwell's hypothesis are the following observations: no negative wave of this type was developed in the schema where the initial development of pressure occurred at some 300 to 350 mm. Hg per second; negative waves were developed in the normal dogs where the initial pressures developed at some 800 to 900 mm. per second but these waves were slight, and usually disappeared again at compression pressures well above the diastolic level; the negative waves were well developed and persisted over a wide range of compression pressure in dogs with aortic regurgitation in which the pressure rise developed at 1100 to 1800 mm. per second; in man the development of these negative waves has been more marked in older subjects, where greater contrasts in pulse wave velocities of the compressed and uncompressed vessel are to be anticipated; the common disappearance of a negative wave at the higher compression pressures might depend on the vessel wall attaining, under these conditions, only a low tension and a relatively low rate of wave transmission.

It was suggested by Bazett and Laplace (1933b) that pulsations could not pass a cuff unless the pressure remained above the compressing pressure long enough to allow the wave to complete the passage of the compressed segment. Erlanger (1916) considered that passage of fluid along the angles of a compressed tube could occur even in arteries. Both these assumptions were found to be untrue.

Accuracy of indirect estimates of lateral pressure. At the lateral systolic level the compression system introduces two errors of opposite sign, one a rise in systolic pressure from a positive reflection of the wave, the other a fall due to an increased distensibility of the system; at this level both of these errors are minimal, and tend to neutralise one another, so that the actual pressure is not much altered. The oscillometric excursion is increased, by the "bolus" type of wave described, at considerably higher

pressures which correspond with end pressures, the lateral systolic criterion appears to be developed at pressures about 5 mm. below the true lateral systolic pressure. A recordable peripheral pulse is developed at an intermediate level, and in these experiments at one which only slightly exceeded the true lateral pressure, but the error of such a criterion is apt to vary with the sensitivity of the recording system, and its ability to demonstrate the presence of the bolus type of wave. The tambour system used on man is slightly more sensitive in this regard than auscultation, while auscultation appears to be more sentisive than a direct recording system. For von Bonsdorff (1932), reviewing the data obtained by Wolff and himself by direct arterial puncture in man, shows that the auscultatory estimates had errors ranging from +13 to −1 mm., with a mean value 5 mm. too high, while estimates from the recurrence of a directly recordable pulse had errors ranging from +15 to −12, with a mean value 1 mm. too high. Part of the considerable discrepancies of von Bonsdorff's data may depend on the fact that simultaneous measurements, when made, involved opposite arms, and that for the most part the comparisons were not simultaneous. Combining von Bondorff's data on man with that here reported on dogs, it would appear that the reappearance of a peripheral pulse, recordable on a tambour system, is likely to have a variable error of some +5 mm. In a large number of estimations on man the differences between this "Sp" estimate and the lateral systolic estimate "S" has averaged 5 mm.; if the Sp criterion is too high, then the S criterion probably has less of a negative error than in the animal experiments, and on a priori reasoning its error is apt to be the less variable. It appears the preferable criterion.

In regard to criteria for diastolic pressure the data presented indicate that the flattening of the end of an oscillometric curve is a poor indicator either of lateral or end diastolic pressure, since its occurrence is modified by the changes in diastolic pressure, which may be produced by compression. On the other hand, the presence of an initial negative wave in the peripheral pulse appears to be quite independent of such variations in the peripheral section, but while its presence is conclusive evidence that diastolic pressure has been exceeded, its absence is less conclusive, since the wave is not always readily developed. Similarly a sharp oscillometric upstroke of maximum steepness is probably an accurate index, but an abrupt development of this change is not always obtained. The ordinary auscultatory criterion (commencement of fading), if deflation is slow, appears to be accurate *on the average* in normal subjects under normal conditions in these experiments, as in those reported by von Bonsdorff, but to be unreliable in other conditions. The criticism by von Bonsdorff of oscillatory estimates based on the size of pulsations is supported; his criticism of estimates based on the shape of waves appears unjustified for the

method he employed appears to have been inadequate to record the initial negative waves.

The work here reported was initiated as the result of attempts to correlate blood pressure changes with stroke volume (Bazett et al., 1934); it is hoped to give a fuller account of these experiments soon. It may be pointed out here that the apparent unwarranted emphasis here given to discrepancies of only a few millimeters between direct and indirect estimates is not artificial. The difference between dicrotic and diastolic pressures in man is often only 15 to 20 mm., so that an error of only 2 mm. in estimating this difference may cause a 10 per cent error in such calculations.

SUMMARY

1. It has been shown in a schema, and also in dogs, that the process of compression of a segment of the vascular system by an air column alters the actual pressures within the system at compression pressures which exceed diastolic pressure. Diastolic pressure is raised particularly at pressures just above the diastolic level; systolic pressure is lowered at pressures below the systolic level, raised at pressures above it. At the true lateral systolic level these two effects more or less neutralize one another. The changes are shown to be modified by the volume of the compressed segment. The raising of diastolic pressure and lowering of systolic result from the decreased volume elasticity coefficient created by the air column; the ultimate rise in systolic pressure depends on the stoppage of the flow and reflection of the primary wave; this second change is only brought gradually into action by an air compressing system, owing to the cushioning effect of the air column.

2. The effects of compression on diastolic pressure may be very unequal in the two separated parts of the main system. When separation is effected, the pressure changes proceed independently in the two sections according to their own volume elasticity coefficients and rates of outflow. The peripheral pulse pressure tends to be the greater owing to the lengthening of the diastolic period in this section, and the consequent fall of diastolic pressure to a lower level, but the whole picture may be altered by the direction in which the contents of the compressed segment are mainly discharged. In general this discharge is towards the side which has the slower rate of fall of pressure.

3. The changes observed on compression in dogs with aortic regurgitation are shown to indicate retrograde flow in the femoral artery during diastole.

4. An oscillometric increase in pulsation is shown to be an accurate indicator of end systolic pressure; a recordable peripheral pulse occurs at pressures above the true lateral systolic level, but owing to the balance of

opposing factors the total error is usually small. The lateral systolic pressure criterion previously described occurs in dogs at pressures somewhat below the true pressure; some reasons are given which suggest that the error involved is even less in man.

5. Dicrotic pressure may be fairly accurately estimated by oscillometric methods under favourable conditions.

6. Diastolic pressure may be estimated by the development of a very rapid upstroke on the oscillogram as the vessel wall assumes a floating position; at the same or at a slightly higher pressure an initial negative wave usually precedes the upstroke of the peripheral pulse, oscillogram or both. These criteria are not affected by changes in the diastolic pressure of the peripheral section. The flattening of the final area of an oscillogram is not a good indicator of either the end or lateral diastolic pressure, as it is affected by changes in the peripheral diastolic pressure produced by compression.

7. The flow of fluid along the angles of compressed arteries is denied; fluid transmitted at compression pressures, which exceed the lateral systolic pressure, does so in the form of a wave of "bolus" type.

We would like to take this opportunity to thank Miss M. E. Maxfield and Mr. N. P. Wander for their assistance in many of the experiments and also our technical assistant Mr. Albert Afford for the skill and care exhibited in making the thin rubber tubing used in some of the experiments.

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THE ERYTHROCYTE AND HEMOGLOBIN INCREASE IN HUMAN BLOOD DURING AND AFTER EXERCISE[1]

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Received for publication March 5, 1935

Regardless of the final outcome of the discussion concerning the explanation of the method of the increase in the number of erythrocytes and the percentage of hemoglobin in a unit volume of blood that occurs during physical exertion, the circumstance under which the increase is brought about and the time taken for its onset and disappearance continue to be of interest to biologists.

We present, therefore, data obtained during and after work on a bicycle ergometer and after the more general exercise of running. Attention has been centered on the variations in the content of red blood corpuscles and hemoglobin in the capillary blood of the fingers. We have used the capillary blood, in spite of the fact that gravity plays a part in the distribution of blood corpuscles. The influence of gravity is one of stagnation, which best reveals itself when the individual stands at attention. Under this condition the capillary blood from the toes is richer in corpuscles than that of the fingers or of the lobe of the ear. The blood of the lobe of the ear contains less than that of the fingers.

Arnold and Krzywanek maintain that the blood should be taken from a vein when rapid blood changes are investigated. Our own experience with blood drawn from a vein and contrasted with that from the skin capillaries is that the two methods, as far as the changes due to physical exertion are concerned, reveal the same facts. The blood drawn from the basilic vein of the arm is on the whole slightly less rich in corpuscles than is that of the capillary blood of the finger of the same arm, but the difference is by no means constant. We have occasionally found the increase in erythrocytes and hemoglobin in men after running fully as great in the venous as in the capillary blood.

The influence of the load of work. The increase in erythrocytes during exertion is regarded as a compensatory adjustment whereby the tissues are more adequately supplied with oxygen. It is assumed by Beyne, Binet and Strumza that, up to a certain limit, corpuscles are supplied by the spleen in response to the demands set up by oxygen want. Since there

[1] The expense of this investigation has been met by a grant from the Charles Himrod Denison Fund.

is a rectilinear relationship maintained between the consumption of oxygen and such factors as the minute volume of breathing, the blood flow, the pulse rate, and other conditions, it seemed desirable to determine if a similar relationship is maintained between the load of work (oxygen requirement) and the increase in erythrocytes in a unit volume of blood. That such a relationship does not exist is shown by the data in table 1. The subject, J. D., worked at well spaced intervals on the bicycle ergometer for five minutes under loads of 4000, 6000, 8000, and 10,000 foot-pounds. With a load of 4000 foot-pounds, which called for approximately 1480 cc. of oxygen per minute, no change in the content of erythrocytes and hemo-

TABLE 1

Work on bicycle ergometer for 5 minutes by J. D.

LOAD	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
foot-pounds	*millions*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
4,000	5.17	5.15	0	116	117	0
6,000	5.16	5.43	5.2	115	120	4.3
8,000	4.98	5.50	10.4	113	124	9.7
10,000	5.16	5.80	12.4	114	127	11.4

TABLE 2

Load of 4000 foot-pounds on bicycle ergometer, C. B. C.

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
minutes	*millions*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
5	5.59	5.62	0	116	116	0
10	5.95	5.80	0	119	118	0
15	6.04	6.10	1	122	124	1.6
20	5.88	6.18	5.1	118	123	4.2

globin could be demonstrated. We had one subject who showed no increase in either erythrocytes or hemoglobin even when carrying a 6000 foot-pound load for 5 minutes. After a load of work is reached that does bring out a response, then each successive increase in load further augments the erythrocyte count (see table 1); but with none of our subjects were we able to show a rectilinear or a curvilinear relationship between the increase of corpuscles and the load of work. The reason for this failure to establish a definite relationship may be found in our next observations.

Time a factor in bringing about the increase. Table 2 shows that a moderate load of work which fails to cause concentration in 5 minutes may do so if continued for a longer period. The load of 4000 foot-pounds was an easy normal-load for this subject (C. B. C.); in fact, any healthy young man

quickly enters and maintains a steady-state in oxygen intake during a long period of work with this load. From the data in table 2, it would seem that some other factor than oxygen want may provoke the increase. It will be observed that after 15 minutes the 4000 foot-pound load had slightly increased both the erythrocyte count and the hemoglobin determination, and that during the next 5 minutes the increase rose to 5 per cent.

Even with an extremely heavy load, such as 10,000 foot-pounds, the erythrocyte increase is somewhat slow in developing. In table 3 is given a typical case, in which at the end of one minute no change had occurred, while at the end of 2 minutes a 7 per cent and at 3 minutes a 13 per cent increase was observed. The 13 per cent increase in erythrocytes in 3 minutes was as large as any obtained with this subject for this load in 5 minutes of work.

TABLE 3

Load of 10,000 foot-pounds on bicycle ergometer, C. B. C.

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
minutes	*millions*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
1	6.09	6.06	0	121	120	0
2	5.99	6.43	7.3	121	128	5.8
3	6.13	6.94	13.2	121	130	7.4

Exertions that call upon more muscles than does pedalling a bicycle ergometer may more rapidly induce an augmentation in the number of erythrocytes. Thus in table 4 are given observations on a man, (H. L.), who twice ran 100 yards in somewhat less than 11 seconds. Blood drawn immediately after the run showed an increase in the count of 7.1 per cent after the first and 9.4 per cent after the second. The runner held his breath throughout the run.

Another series of runs of 100 yards made by M. R. W. (table 4) indicates that the more strenuous the effort the sooner and the greater will be the rise in the erythrocyte count. When M. R. W. ran the distance in 16 seconds no change was found immediately after the run, but when he lowered his time to 14 seconds an erythrocyte increase of 1.8 per cent was obtained, and after he ran the 100 yards in a little less than 13 seconds the augmentation rose to 6.9 per cent.

By means of violent "running in place" in the laboratory it was demonstrated that the augmentation in the number of erythrocytes may develop some time after the exertion is over. There were 8 men who ran 14 times for 30 seconds. For these the first sample of blood, drawn within 15 to 30 seconds, gave variable results, 4 showing an increase and 8 a decrease in

the count. Of the 4 who gave the increase we may conclude that it was real, since the second samples showed a further augmentation. Of the 8 who had a decrease 4 were decidedly beyond our probable errors, showing a drop of from 5.5 to 9.6 per cent. This drop appeared to be associated with a cutaneous vasoconstriction. When this vasomotor condition passed, as it always did by the time for the second sample, the increase of erythrocytes became clear. The time of maximum increase was usually delayed beyond one minute; in 2 instances it occurred at end of the third minute, while in 6 men it occurred between the second and third minute. The data of two typical runs are given in table 5.

TABLE 4

100-yard run

TIME	RED BLOOD CELLS			HEMOGLOBIN		
	Rest	Exercise	Increase	Rest	Exercise	Increase
			M. R. W.			
seconds	*millions*	*millions*	*per cent*	*per cent*	*per cent*	*per cent*
16	5.60	5.59	0	122	123	0
14	5.60	5.70	1.8	122	125	2 5
13	5.40	5.77	6.9	115	122	6 1
			H. L., held breath			
11	5.48	5.83	7.1	126	135	7 1
10.7	5.43	5.94	9.4	124	134	8 1

TABLE 5

After 30 seconds of "running in place"

SUBJECT	RED BLOOD CELLS IN MILLIONS										
	Norm.	30"	60"	1' 45"	2' 15"	3' 15"	4' 15"	5' 15"	6' 15"	7' 15"	8' 15"
N. M.....	5.40	5.37	5.86	6.24	6.34	5.60	5.44	5.53	5.18	5.24	5.21
R. B.....	5.09	5.43	6.19	6.32	5.38	5.45	5.46	5.31		5.17	5.26

"Running in place" violently for 15 seconds also gave results that corresponded with those of the 30-second runs. Usually the increase in erythrocytes develops in the post-exercise period; but in exceptional cases it is well developed in the first blood sample, drawn within 15 seconds after the exertion. In one instance the maximum concentration was obtained in the first sample. The maximum rise in the erythrocyte count after the run of 15 seconds was less than after the 30-second run and ranged from 6 to 8 per cent. In every case this was reached before or by the end of the second minute.

After the 30-second run the maximum erythrocyte increase for all cases ranged between 6 and 19.5 per cent, with 10 out of 14 cases above 11 per cent. After all of the 30-second runs the men were badly "winded." On the average the augmentation in the erythrocyte count after the 30-second run only slightly exceeded that obtained with the heaviest load on the bicycle ergometer during a period of 5 minutes of work. The highest for this load was an increase of 19.3 per cent, while the maximum for the majority ranged between 10.5 and 12 per cent.

Time of recovery. The duration of the period of high concentration of the number of erythrocytes was short after the 15-second runs, usually not over 3 minutes. After the 30-second runs the majority of cases required in the neighborhood of 10 minutes to return to the pre-exercise level. In one man, in whom the maximum occurred one minute after this exercise, the return to normal was made in 3 minutes, but all of the others required 5 minutes or more. After the heaviest load, 10,000 foot-pounds, on the bicycle ergometer the normal count was not restored in less than 6 minutes and usually required 8 or more minutes. We did not ordinarily continue sampling beyond 10 minutes.

SUMMARY

During 5-minute periods of work on a bicycle ergometer the number of erythrocytes and the percentage of hemoglobin in a unit volume of blood were not increased with light loads. With heavier loads of work an increase invariably occurred and augmented as the load of work was stepped-up.

No mathematical relationship between the increase in erythrocytes and hemoglobin and the load of work was established.

A very moderate load of work, if carried long enough, may eventually, within 15 or 20 minutes, induce an increase in the number of erythrocytes. This suggests that some other factor than anoxemia may operate to induce the concentration.

Exertions that call generally on the muscles of the body, such as in running, more promptly induce the augmentation in erythrocytes. The increase was observed immediately after a 100 yard run in 11 seconds.

Ordinarily after violent "runs in place" of 15 and 30 seconds the maximum degree of concentration is slowly reached, usually within from 2 to 3 minutes.

The time required for return to normal after running is determined in part by the duration of the effort. This was accomplished within 3 minutes after 15-second runs and required in the neighborhood of 10 minutes after the 30-second runs.

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DISTRIBUTION OF VAGUS CONTROL OF THE TURTLE HEART

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Received for publication February 5, 1935

Differences in the action of the right and left vagi upon the turtle heart have been frequently described (e.g., Gaskell, 1883–84; Garrey, 1911). It is generally concluded that different fibers of the vagus exert different effects upon rhythm, contraction, conduction, and excitation; these effects have been described as chronotropic, inotropic, dromotropic, and bathmotropic, respectively, by Englemann (1900). Two possibilities offer themselves: 1, there may be four different types of fibers mediating four different mechanisms; 2, a single mechanism may be mediated by fibers which are distributed to different regions of the heart. This question is of particular interest with regard to the humoral hypothesis of vagus action (Loewi, 1921), since, according to this concept, a single vagus substance would be expected to produce various effects. The following studies were undertaken with this particular question in mind.

EXPERIMENTAL. In our first experiments we attempted to study the distributions of inotropic and chronotropic effects between the two vagi. The turtles used were of two species, namely, *Pseudemys elegans* and *Clemmys marmorata*. The contractions of both auricles were recorded simultaneously upon a kymograph by means of light heart levers of equal magnification, these being attached to the apices of the auricles by light threads. The vagi were dissected out in the neck, ligated, and stimulated through platinum electrodes by an interrupted current from a Harvard inductorium.

In the species *Pseudemys elegans* the right vagus caused cardiac standstill with moderate strength of stimulation in three out of four experiments; in the fourth experiment standstill resulted with strong stimulation. The left vagus produced only a slight slowing in three cases with all strengths of stimulation. The results with the second species, *Clemmys marmorata*, were similar to those mentioned above, but in five cases out of six strong stimulation of the left vagus caused complete cessation of the beat in a manner similar to the right vagus. Thus there appears to be a considerable difference between species in the distribution of fibers mediating chronotropic effects.

Since the stimulation of the right vagus usually stops the rhythm of the entire heart, any effects upon the amplitude of contraction which might take place during the stimulation are masked by the destruction of this rhythm. Accordingly, it was necessary to provide some means by which contractions could be maintained during right vagus stimulation. To accomplish such a result a Gaskell heart clamp with jaws protected by means of adhesive tape was placed at the sino-auricular junction and pressure gradually applied at this point. When the pressure was sufficiently great, the auricles ceased to respond to the impulses arriving from the pacemaker, and rhythmically occurring single induction shocks were then applied to the ventricle at rates approximating the normal rhythm. These were obtained from a second inductorium, whose primary circuit was made and broken at regular intervals by a rotary contact maker. These shocks caused regular contractions of the ventricle, followed by contractions of both auricles. Thus a ventricular-auricular rhythm was maintained. It has been demonstrated by Garrey (1911) that compression at the sino-auricular junction in the turtle does not destroy the vagus fibers, although preventing conduction through the tissue itself; and it was thus possible to observe any effect upon the contraction of the auricles during vagal stimulation. Previous to the clamping off of the sino-auricular junction, observations were made as to any changes on the rate of the normally beating heart which might be produced by either vagus.

In both species, under the above conditions, stimulation of the right vagus caused a diminished amplitude of contraction or negative inotropic effect in both auricles; figure 1 illustrates this clearly. Figure 2, which is the record of the same heart beating at its own rhythm, demonstrates the fact that ordinarily during right vagus stimulation such an inotropic effect is masked by the cessation of the rhythm. This is especially marked in the left auricle where the first contractions are actually greater in amplitude upon resumption of the beat, probably due to the greater recovery period during quiescence (see Blum, 1927).

Distribution of the vagi to the two sides of the heart appears somewhat variable. In four individuals of species *Pseudemys elegans*, the stimulation of either the right or left vagus caused a diminution of contraction which was greater in the homolateral auricle. This occurred even in the case of fairly weak stimuli. In species *Clemmys marmorata* the results were more varied. In three of four animals the right vagus produced a greater diminution of contraction in the homolateral auricle, but in the fourth case the contralateral diminution was greater than the homolateral. The stimulation of the left vagus in two of the four experiments, produced a greater contralateral diminution of contraction, but the remaining cases showed the homolateral effect of the left vagus to be the greater.

Although the total number of our experiments is not great, the results

on the artificially driven hearts show that, generally, the negative inotropic effect of each vagus is greatest on the auricle on the same side as the nerve stimulated, as observed by Garrey (1911) and Cruickshank (1920, 1921). However, it must be noted that in eight experiments there were three exceptions to this generalization, and we may therefore assume that variations are common. A comparison of the inotropic effects of both nerves indicates that the left nerve appears to exert on its own side a greater negative inotropic effect than that exerted by the right vagus on either side.

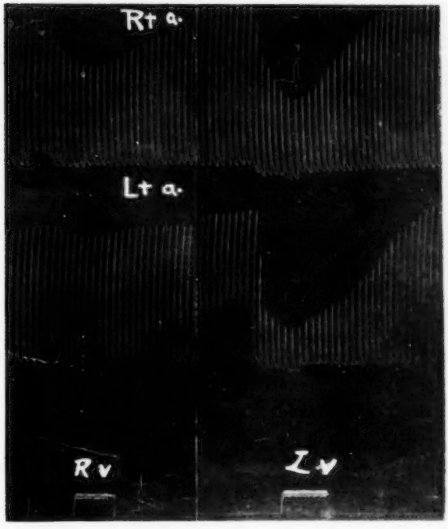

Fig. 1

Fig. 2

Fig. 1. Contractions of artificially driven turtle auricles (*Clemmys marmorata*). *Rt. a.*, right auricle; *Lt. a.*, left auricle; *R. v.*, stimulation of right vagus; *L. v.*, stimulation of left vagus.

Fig. 2. Contraction of turtle auricles following normal rhythm from the sinus. *Rt. a.*, right auricle; *Lt. a.*, left auricle; *R. v.*, stimulation of right vagus; *L. v.*, stimulation of left vagus.

An example of this may be seen in figure 1. This must be cautiously considered, however, since it is possible that the pressure of clamping the sino-auricular junction might have caused a partial injury to the vagus fibers, particularly those of the right vagus. However, it can be seen from an inspection of figures 1 and 2 that in the case of the left vagus there was no apparent injury to the vagal fibers during the application of the clamp as judged by the magnitude of the inotropic effect of the left vagus before and during artificial rhythm.

In a second group of experiments, the threshold of stimulation of the chronotropic and inotropic effects in the two vagi were studied after the method of Gilson (1930). The species employed in these studies was *Clemmys marmorata*. A neon tube oscillator, whose output and rate of oscillation could be controlled, was employed for stimulating the vagi; the particular circuit has been described by Lange (1931, 1932). The stimulating rates most frequently used varied between six and fourteen shocks per second.

In these experiments the difference in the actions of the right and left vagi on the beat of the sinus and upon conduction over the sino-auricular junction was first studied. Each vagus was stimulated separately with currents first below the threshold of any effect and gradually increased to maximum output of the machine; the changes in rate and sino-auricular interval which were apparent by direct observation of the beating heart were noted in the order in which they appeared. A few attempts to make kymographic records of the contractions of the sinus showed that the act of attaching threads to the sinus in some way interfered with the inhibition of the pacemaker mechanism, since direct observation of changes in the contraction rate differed from the changes recorded when the sinus was attached to a lever. Furthermore, the vagal chronotropic effects were not so pronounced, nor were sino-auricular interval changes so evident.

Twenty-one different animals were examined in this group. In approximately half of the vagus nerves the chronotropic and dromotropic effects had identical thresholds; i.e., as the current was gradually increased, the decrease in the rate of sinus beat, and increase in sino-auricular interval occurred simultaneously. In the other half of the nerves the chronotropic effect had a lower threshold except in two instances, both left vagi, in which the dromotropic effect had the lower threshold. In two of these it was possible to produce slowing of the sinus beat with the maximum current output of the apparatus.

The lengthening of the sino-auricular interval was usually followed by sino-auricular block, thus producing a greater *apparent* chronotropic effect in the auricles and ventricles than in the sinus.

In a majority of cases the sinus could be brought to complete standstill; this was more common with the right than with the left vagus. An interesting phenomenon discovered was the fact that, although the pacemaker could be completely inhibited by either vagus so that the whole heart was quiescent, a mechanical stimulus applied to the sinus would cause that organ to contract, and if inhibition had not interfered with conduction this contraction would be followed by the rest of the heart in sequence. This indicates that conduction over the whole heart is still possible in spite of the vagus action.

Additional experiments were performed to test the action of stimulating

currents of various strengths on the inotropic and chronotropic effects produced in the auricles. In the first few experiments the beat of only one auricle was recorded. However, since the inotropic action of either vagus is greatest on the homolateral side, the homolateral auricle should be the better index of any changes in contractility during the stimulation of the vagi, and for this reason in the latter experiments the contractions of both auricles were recorded. The procedure followed was similar to that used above. While one or both auricles were recording on a kymograph by means of light isotonic heart levers, the vagus nerves were stimulated separately with sub-threshold currents. Next the strength was increased until either inotropic or chronotropic action occurred. Then the stimulus was increased until no further depression of contractility or rate was noted in the auricles.

Eight different animals were utilized in these experiments. In all but two nerves the inotropic effect had the lower threshold on either the homolateral or contralateral auricle. Both exceptions to this general rule occurred in right vagi. In one case the threshold for the chronotropic effect on the homolateral auricle was lower than the inotropic effect; unfortunately the contralateral auricle was not studied in this animal. In the other exception, the thresholds were identical for the inotropic and chronotropic effects in the contralateral auricle. In only one animal of this series did the left vagus fail to produce a chronotropic effect.

Discussion. The results show that in the majority of cases, with sufficient strength of stimulating current, the right vagus can stop the beat of the sinus. A negative inotropic action in the sinus and caval veins is often associated with this decrease of the rate of beat, but it must be noted that the cessation of the beat is not due to the decline of the sinus contractility to zero, for in many experiments it has been possible to show that the sinus will still contract when stimulated mechanically, although the pacemaker mechanism is under complete inhibition by the vagus.

The results of the stimulation of the left vagus on sinus rate are similar to those just mentioned, although the number of experiments in which the left could stop the heart was not so great; particularly in *Pseudemys elegans*. Examination of the left caval veins indicated that the contractility of those parts was strongly diminished during the stimulation of the left nerve.

Both vagi can influence sino-auricular conduction as is evidenced by a lengthening of the sino-auricular interval. This lengthening gradually gives way to partial block where the sinus beats at a regular rate though somewhat slower than normal, followed by every other beat of the auricles. Occasionally, it is possible to adjust the current strength of the stimulator so that complete block will take place. In one experiment with the left

vagus, it was found possible to produce complete block without any apparent decrease in rate of beat of the sinus. This single result is similar to those reported by von Skramlik (1932) in *Testudo graeca*, who concludes that this nerve does not innervate the pacemaker for the heart, since the left vagus could only produce slowing of the auricles and the ventricles by block at the sino-auricular junction and not by influencing the rate of the pacemaker. This may be the case in the species in question, but in our experiments on *Clemmys marmorata* it was the exception rather than the rule.

The results on the chronotropic and inotropic effects, as indicated by auricular contraction records, agree with those of Gilson (1930) and Heinbecker (1931) with two exceptions, one where the chronotropic effect appears to have a lower vagal threshold than the inotropic and one where the thresholds are identical. It must be noted that there is generally an overlap between these two effects. That is, beyond the chronotropic threshold, there is still greater reduction in auricular contractility or negative inotropic effect. Heinbecker also refers to this overlap, but concludes that in spite of such a fact, "it seems not inconsistent" to consider that the inotropic mechanism is innervated by a certain set of fibers and the chronotropic by an other type. However, this overlapping and the one exceptional case of a lower chronotropic threshold in the right vagus which we have found in our work are hard to reconcile with the concept of inotropic and chronotropic fiber types. In any event the question of the existence of distinctive inotropic and chronotropic fibers remains without definite answer.

CONCLUSIONS AND SUMMARY

The distribution of function of the right and left vagus nerves shows considerable variation in the two species of turtle examined. Such variations may have an important bearing on the interpretation of experimental results. Chronotropic and inotropic effects can be separated to a certain extent on the basis of stimulation thresholds of the vagus trunks but a definite overlapping exists. We have been unable to separate chronotropic and dromotropic effects on the basis of thresholds. It would thus seem that no definite evidence can be brought forward against the concept that a common mechanism is mediated by the vagus fibers and that the different effects produced are the result of the distribution of these fibers.

Our results, like those of Cohn (1912) on the dog, would appear to indicate that there exists a wide variation in the distribution of vagus fibers to the heart; this offers great difficulty in experimental work on the innervation of this organ. We may assume that the integrated control of this organ is mediated by numerous fibers producing chronotropic, inotropic, or

dromotropic effects according to the regions of the organs which they innervate, but the part played by these various effects in the economy and function of the organ remains obscure.

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STIMULATION OF THE HYPOTHALAMUS WITH SPECIAL REFERENCE TO ITS EFFECT ON GASTRO-INTESTINAL MOTILITY[1]

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Received for publication February 20, 1935

A series of investigations is being conducted in this laboratory on the vegetative responses to electrical stimulation of the hypothalamus. The present report is concerned with the gastro-intestinal responses. Ranson and Magoun (1) reported increased rate and amplitude of respiration and marked dilatation of the pupils from stimulation of the lateral hypothalamic area and later work (2, 3) showed that a rise in blood pressure, contraction of the bladder and frequently struggling could be obtained from the same region. The above experiments were done under nembutal anesthesia, which is known to depress intestinal motility (4). To avoid such effects, the present experiments were conducted in the waking animal.

It should be emphasized that the hypothalamus is a very small region at the base of the diencephalon on either side of the third ventricle, comprising, in the cat, a cube each dimension of which is approximately 4 mm. There are a number of distinct nuclei, some having efferent connections with the posterior and intermediate lobes of the hypophysis, others sending impulses caudally to lower visceral centers. This region is very difficult to reach by ordinary methods. The difficulty has in great measure been overcome by the use of the Horsley-Clarke stereotaxic instrument to orient the electrodes. With the aid of this apparatus, it is possible to place the tip of the electrode within reasonable limits at any desired point in the interior of the brain and to stimulate a localized area of brain tissue immediately surrounding that point. The point is located by using the millimeter scales on the instrument, the coördinates being known from a study of serial sections of normal cat brains.

METHODS. Twenty-nine cats were used. The animal was anesthetized with ether, the calvarium exposed, and the Horsley-Clarke instrument adjusted to its head. The details of the construction of the instrument and of its use have been described by Horsley and Clarke (5) and Ranson (6).

[1] Aided by a grant from the Rockefeller Foundation.

A point on the surface of the skull was located by means of Horsley-Clarke coördinates and a small trephine hole was made. A metal cuff of the same diameter was screwed into the hole to the point where it was held firmly by the bone and exerted a slight tension on the dura below.

With the aid of the instrument, the electrode was put down to the desired point in the brain. The bipolar electrode was made of three lengths of enamelled nichrome wire cemented together, two fine wires (28 gauge) and a heavy wire (22 gauge) for support. Insulation covered all of the heavy wire and all of the fine wires except the tips of the latter, which were separated by about 1 mm. along the axis of the electrode. In three cats, special electrodes which had four fine wires cemented around a supporting wire were used, only the tips of the fine wires being uninsulated. The ends of two fine wires, separated by 1 mm., extended to the tip of the electrode, while the ends of the other two wires, also separated by a millimeter, only extended to within 8 to 10 mm. of the tip. With this type of electrode, two points, one more dorsally placed than the other, could be stimulated in the same cat.

In every case preliminary stimulation was conducted to determine from the response whether the electrode was at the desired point. From one to ten punctures were made, usually less than five, and several points were stimulated along each puncture. The method of exploration is described in detail by Ranson and Magoun (1). Once the electrode was properly oriented, it was left in place. An additional electrode was inserted in six of the cats, in one instance with the aid of the instrument, in others by hand; in three cats, the special electrodes described above were used, making possible the stimulation of two points in each brain.

Then the dura was carefully dried and dental cement was packed around the electrode and the cuff. When the cement was hard, the instrument was removed from the cat's head and the supporting wire cut level with the cement. The skin wounds were clipped and the animal was bound, lying on its side, in a comfortable hammock. After this the anesthesia was stopped. Not until two and one-half hours later, on the average, by which time the cat had fully recovered from the anesthesia, was the barium meal administered by stomach tube, and fluoroscopic observation of the alimentary tract begun.

All radiographic observations were made with a standard Victor 5 inch 30 M stabilized x-ray apparatus, supplied with a Coolidge 30 MA tube; a milliamperage of 6 was uniformly employed. During the progress of the observations several short exposures were made within the predetermined interval between peristaltic waves, in order that not even minor changes in the digestive tube would escape attention. At all times during exposure the outline of the barium meal was remarkably clear.

Observations were made of the peristaltic movements of the stomach

and intestine and the number of gastric waves initiated in one minute were counted. Faradic stimulation, of intensities varying from below threshold to strengths sufficient to give fully developed reactions, was then applied. The response of the gastro-intestinal tract was noted and latent period and after-effect were carefully recorded. To facilitate the observation of other responses to the stimulus, both vegetative and somatic, the lights were then turned on, preventing simultaneous visualization of the gut. This procedure was repeated a number of times, varying the coil distances and allowing time between stimuli for recovery.

After sufficient data had been collected, the animal was killed with ether, and in some cases the mucous membrane of stomach and small intestine was examined. The head was then injected with formalin and when the brain was hard, the bone was cut around the cuff and the electrode pulled out vertically with great care. The brain was removed and a block containing the region stimulated was embedded and cut into transverse sections, which were stained by Weil's method. The space that the electrode had occupied was easily identified and the point stimulated was located on a drawing of a transverse section of the brain stem.

Results. Despite the operation, punctures and electrode in the brain, and frequent stimulation, gastro-intestinal movements between stimuli were as vigorous and as rapid as in normal controls. The cats would usually lie quietly, sometimes purring. In the animals which cried out and struggled occasionally, peristalsis was not disturbed. In two cats, however, one of which was a pregnant female, no peristalsis was seen during the entire period of observation. The electrode in both cases pierced the infundibular stalk, one lodging in the anterior lobe of the pituitary gland. The experimental animals were as variable as normal ones in regard to the time between feeding and the first appearance of peristalsis or the first passage of meal through the pyloric sphincter, the site of the electrode apparently not being a factor.

The points stimulated have been classified according to location and will be discussed in the following order:

A. Hypothalamus—8 points.

B. Very close to hypothalamus—2 points.

C. Septum pellucidum, preoptic area and anterior commissure—11 points.

D. Internal capsule and basis pedunculi—6 points.

E. Infundibular stalk and its environs—6 points.

F. Two points stimulated in the same brain—9 cats.

A. Hypothalamus. The response of the gastro-intestinal tract to stimulation of the hypothalamus was an immediate inhibition of peristalsis and complete loss of tone. The latent period for abolition of gastric waves was very short, approximately one second. If stimulation was begun when a peristaltic wave was halfway along the stomach wall, the wave

stopped abruptly and never reached the sphincter. If the wave was very close to the sphincter when stimulation was initiated, some meal was ejected into the duodenum, but instead of passing rapidly through the latter as is normally the case, the small mass remained stationary at its upper end. The latent period for cessation of intestinal movements was equally short. Loss of tone came on a little more slowly, the stomach losing its constrictions and becoming baglike, the intestine losing its beading and becoming dilated and ribbon-like. The pyloric sphincter may have been contracted, for the rapid, deep respiration and frequent violent struggling which occurred during stimulation could probably raise intra-gastric pressure sufficiently to discharge some meal through a relaxed sphincter. The inhibition was maintained during stimulation and for a variable period, usually about 30 to 90 seconds, after termination of the stimulus. This after-inhibition may be due to sympathetic after-discharge or to a secretion of adrenalin.

Accompanying the gastro-intestinal inhibition were marked dilatation of the pupils, greatly increased rate and amplitude of respiration, and erection of hair.[2] Frequently vigorous struggling occurred. Sometimes the head was raised when stimulation was begun and the cat looked from side to side excitedly and very often the claws were protruded, the eyes opened wide and the nictitating membranes retracted. Urination occurred during stimulation in several experiments and in one case rhythmic spitting was observed.

Microscopical examination of the brains showed that in eight of the cats, the tip of the electrode was in the hypothalamus (fig. 1). The exact points stimulated and the threshold coil distances for inhibition of peristalsis were:

Cat 1—in lateral hypothalamic area, 1 mm. from base, lateral to rostral end of mammillary body. Inhibition at coil 20.

Cat 2—in lateral hypothalamic area, 2½ mm. from base, lateral to rostral part of nucleus hypothalamicus posterior. Inhibition at coil 12.

Cat 3—in lateral hypothalamic area, 1 mm. from base, lateral to caudal part of nucleus hypothalamicus posterior. Inhibition at coil 15.

Cat 5—in lateral hypothalamic area, ½ mm. from base, lateral to caudal part of nucleus hypothalamicus ventromedialis. Inhibition at coil 14 (fig. 1).

Cat 6—at junction of lateral hypothalamic area and field H_2 of Forel, 1½ mm. from base, lateral to middle of mammillary body. Inhibition at coil 15.

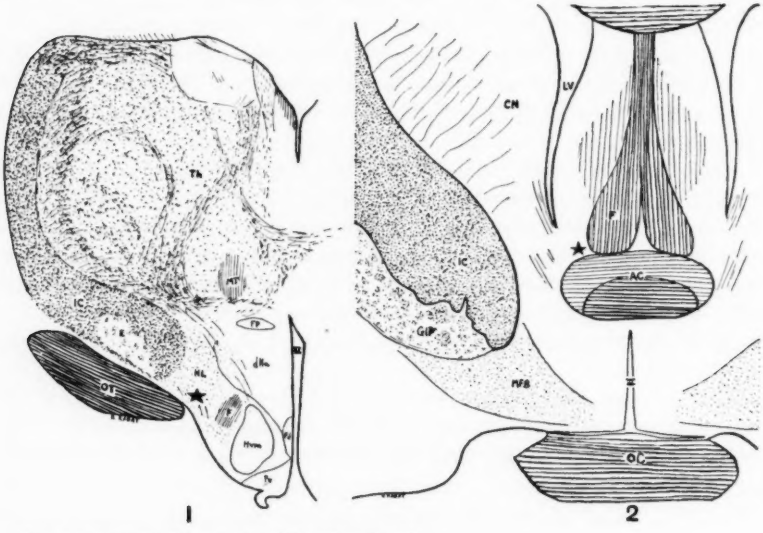
Cat 21—in rostral part of nucleus hypothalamicus ventromedialis, 2 mm. from base, 1 mm. medial to fornix. Inhibition at coil 14.

Cat 26—in lateral hypothalamic area, 1 mm. from base, lateral to middle of mammillary body. Inhibition at coil 14.

Cat 29—in supramammillary commissure in midline, dorsal to middle of mammillary body. Inhibition at coil 15.

[2] This group of responses will henceforth be designated, for want of a better term, as "sympathetic discharge," although the respiratory movements are performed by skeletal muscles innervated by somatic nerves.

All of these points were in the lateral hypothalamic area except two, one of which was in the supramammillary commissure, the other in the nucleus hypothalamicus ventromedialis. The fact that inhibition was obtained from stimulation of the supramammillary commissure may be explained by unpublished observations indicating that impulses from the lateral hypothalamic area cross in part in this commissure. There is a possibility that the inhibition obtained from stimulation of the ventromedial nucleus may not have been due to stimulation of this nucleus but to the spread of current to the lateral hypothalamic area. The assumption that such spread



Stars represent the exact points stimulated.

Fig. 1. Transverse section through the diencephalon of the cat at about the middle of the hypothalamus. Stimulation of the lateral hypothalamic area.

Fig. 2. Transverse section through the telencephalon at the level of the crossing of the anterior commissure. Stimulation of the dorsal surface of the anterior commissure.

occurred is borne out by the observation that inhibition was never obtained from this nucleus unaccompanied by rhythmic spitting. The latter response is localized in the rostral part of the lateral hypothalamic area, in the supraoptic region, about $\frac{1}{2}$ mm. from the point in the ventromedial nucleus.

If the coil distance was too great to produce cessation of peristalsis, stimulation had no effect on tone or motility, but did, however, result in a moderate sympathetic discharge. At the threshold for the gastro-intestinal response, the inhibition was irregular. Sometimes under these conditions peristalsis would stop on applying the stimulus but would appear

again at the normal rate before stimulation was ended. Occasionally no inhibition occurred until some time after the beginning of stimulation. Furthermore, a threshold stimulus that was effective in stopping peristalsis was often ineffective on repetition.

The relation of struggling to the inhibition is of interest. Spontaneous struggling occurred frequently in several animals with no inhibition of peristalsis. During stimulation of the hypothalamus, struggling was sometimes observed without inhibition. When the cat responded to the stimulus with violent struggling, together with a marked sympathetic discharge, cessation of peristalsis always supervened; but the struggling was not essential to the inhibition, since definite inhibition has been observed with no struggling at all and inhibition was often evident long before the onset of struggling.

In some of these cats, the mucous membrane of stomach and small intestine was examined and no hyperemia, hemorrhage or ulceration was seen.

B. Points close to hypothalamus. Cessation of peristalsis was observed in two other cats at coil distances very similar to those that were threshold for the hypothalamus. The tips of the electrodes were found, when the sections were studied, very close to the lateral hypothalamic area:

Cat 4—at base of brain, 1 mm. lateral to mammillary peduncle, about ½ mm. caudal to mammillary body. Inhibition at coil 13.

Cat 27—in field H_1 of Forel of subthalamus, 3 mm. from base, 2 mm. from midline, at level of caudal part of mammillary body. Inhibition at coil 15.

The sympathetic discharge accompanying the inhibition in cat 4 was similar to that obtained from stimulation of the lateral hypothalamic area. Stimulation of the subthalamus, besides eliciting a sympathetic discharge, caused extension of the ipsilateral limbs and flexion of the contralateral limbs.

C. Septum pellucidum, preoptic area and anterior commissure. As a control measure, eleven points were stimulated in the septum pellucidum, preoptic area and anterior commissure in nine cats (fig. 2). No change in peristalsis was observed on stimulation of these regions, using stimuli of a strength that would yield immediate inhibition from the lateral hypothalamic area. Careful study revealed no change in the rate of gastric peristalsis or in the tone of the stomach and intestine. Stimulation was frequently effective in producing urination, slight dilatation of the pupils, slowing or slight acceleration of respiration and in some cases rhythmic crying. The lack of excitement and struggling formed a striking contrast to the vigor of the hypothalamic response.

No gastro-intestinal response was observed, as the secondary coil was approximated to the primary until there was evidence of spread of current

to the hypothalamus when cessation of peristalsis supervened. A marked sympathetic discharge and in three cases also crying accompanied the inhibition of the gut. In one cat stimulated in the septum, a more moderate response accompanied the inhibition—the respiration was greatly accelerated but there was no struggling or erection of hair and only slight dilatation of the pupils.

The coils had to be much closer together for inhibition of peristalsis to result from stimulation of the septum, etc., than from stimulation of the lateral hypothalamus. The characteristics of the inhibition, the latent period and after-inhibition were the same as have been described for the latter region. The location of the various points and the threshold coil distances for inhibition follow:

Cat 7—in medial preoptic area in midline, ¾ mm. rostral to crossing of anterior commissure. Inhibition at coil 10.

Cat 8—in septum pellucidum, ¾ mm. rostral to crossing of anterior commissure near tip of lateral ventricle. Inhibition at coil 7.

Cat 8—(second electrode)—in anterior commissure at its crossing, 2 mm. from midline. Inhibition at coil 4.

Cat 11—(second electrode)—at base of brain, 1 mm. from midline, at level of crossing of anterior commissure. Inhibition at coil 9.

Cat 15—in medial preoptic area in midline, ¾ mm. rostral to crossing of anterior commissure. Inhibition at coil 10.

Cat 15—(second electrode)—at base of brain, 5 mm. from midline, ¾ mm. rostral to crossing of anterior commissure. Inhibition at coil 9.

Cat 16—On dorsal surface of anterior commissure at level of its crossing, 1½ mm. from midline. Inhibition at coil 9½ (fig. 2).

Cat 17—in bed-nucleus of anterior commissure, ¾ mm. rostral to the commissure. Inhibition at coil 12½.

Cat 18—in bed-nucleus of anterior commissure, ¾ mm. rostral to the commissure. Inhibition at coil 11½.

Cat 19—in septum pellucidum in midline, 1½ mm. rostral to crossing of anterior commissure. Inhibition at coil 9.

Cat 20—in interbulbar component of anterior commissure, at its crossing, 1 mm. from midline. Inhibition at coil 9.

D. Internal capsule and basis pedunculi. In five cats, the corticofugal motor fibers were stimulated. The tip of the electrode was found, on examination of the brains, in the internal capsule rostral to the hypothalamus in four cases (fig. 3), while in the other it was located in the midbrain, at the lateral edge of the basis pedunculi. Stimulation of these points, using coil distances that would evoke inhibition from the lateral hypothalamic area, had no effect on peristalsis. These stimuli did result in moderate dilatation of the pupils, some acceleration of respiration, rhythmic lapping and movements of the contralateral forelimb. As the secondary coil was moved closer to the primary, movements of other muscle groups were added to the list. At the threshold for inhibition of the

digestive tract, a sympathetic discharge occurred besides vigorous chewing or lapping, flexion of contralateral and extension of ipsilateral limbs, contraction of neck muscles and occasionally of facial muscles. The inhibition was the same as in the preceding groups of experiments, except in cat 13, in which inhibition did not set in until two shallow waves were completed. No data were collected on after-inhibition. A peculiar phenomenon was recorded in cat 14. In this animal, inhibition of the stomach occurred three times on stimulation at coil 12. The intestine was inhibited only at first, for movements resumed during stimulation. Later stimulation at coil 12, $11\frac{1}{2}$, 11 and 10 was ineffective in stopping peristalsis in the

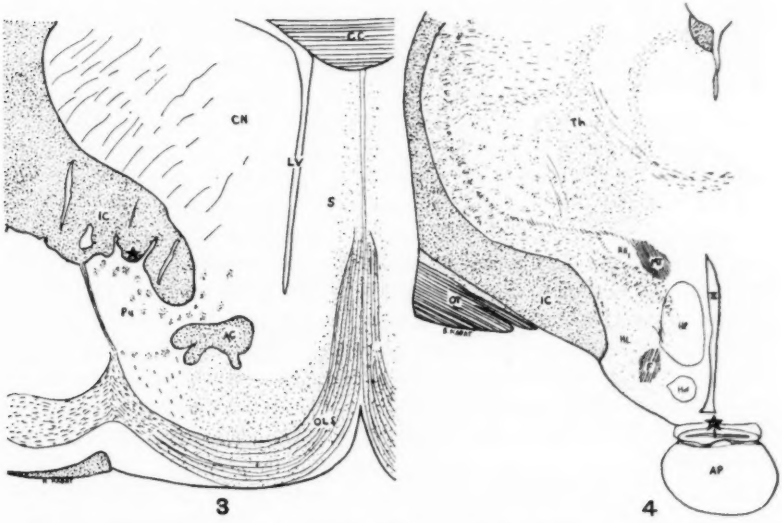


Fig. 3. Transverse section through the telencephalon in front of the crossing of the anterior commissure. Stimulation of the internal capsule.

Fig. 4. Transverse section through the diencephalon at about the middle of the hypothalamus. Stimulation of the infundibular stalk.

stomach or intestine and complete inhibition did not appear until stimulation was carried out at coil $9\frac{1}{2}$.

The exact location of the points stimulated and the threshold for inhibition were as follows:

Cat 10—at lateral edge of basis pedunculi, about 1 mm. caudal to mammillary body. Inhibition at coil 10.

Cat 10—(second electrode)—in internal capsule dorsal to lateral geniculate body. No inhibition at coil 0.

Cat 11—at ventral edge of internal capsule, 2 mm. from its medial border, $\frac{3}{4}$ mm. rostral to anterior hypothalamic area. Inhibition at coil 10.

Cat 12—in internal capsule, 1 mm. from its medial border, among anterior commissure fibers traversing the internal capsule. Inhibition at coil 6.

Cat 13—in external capsule ½ mm. ventrolateral to internal capsule at a level 2½ mm. rostral to crossing of anterior commissure. Inhibition at coil 8.

Cat 14—in ventral part of internal capsule, 1 mm. from its medial border, 2 mm. rostral to crossing of anterior commissure. Inhibition at coil 12 and 9½ (fig. 3).

E. Infundibular stalk. An attempt was made in six cats to stimulate the infundibular stalk, in order to activate the hypothalamico-hypophyseal mechanism but in only two cases was the electrode properly placed for this purpose (fig. 4). Stimulation of the stalk in these two animals and of points near the stalk in two others using coil distances effective in stopping peristalsis on stimulation of the lateral hypothalamic area, produced no change in motility or tone of the alimentary tract; no increase or decrease in activity was ever observed. Such stimulation did, however, cause a slight dilatation of the pupils, a slight acceleration of respiration; and in one case urination, but the animal remained quiet, showing no signs of excitement. After numerous stimuli, gastric peristalsis, as measured by the number of waves initiated per minute, was no more marked than at the beginning of the experiment. When the current was increased sufficiently in cats 22, 23, and 25, stimulation of the stalk resulted in cessation of peristalsis, along with vigorous spitting and crying, urination, and a marked sympathetic discharge. In cat 14, the same response occurred without spitting or crying. Somewhat more moderate sympathetic and somatic discharges took place on stimulation at coil distances too great to inhibit the gut. The fact that in two cats with electrodes in this region there was no peristalsis at all seen during the experiment has already been mentioned.

The gastric and intestinal mucous membrane in each of these animals was examined at the end of the experiment and no hyperemia, hemorrhage, or ulceration were discovered.

A microscopical study of the brain revealed that the tip of the electrode was successfully oriented in two of the cats. The points stimulated and the thresholds for inhibition were as follows:

Cat 14—(second electrode)—out of brain, about ½ mm. ventrolateral to middle of mammillary body. Inhibition at coil 10.

Cat 22—at base of brain, ½ mm. lateral to infundibular stalk, at level of rostral part of nucleus hypothalamicus posterior. Inhibition at coil 9½.

Cat 23—out of brain, about ¾ mm. ventral to infundibular stalk in midline, in front of pituitary. Inhibition at coil 9½.

Cat 24—in anterior lobe of pituitary. No peristalsis seen.

Cat 25—in infundibular stalk in midline at level of posterior hypothalamic nucleus. Inhibition at coil 11 (fig. 4).

Cat 28—through infundibular stalk in midline, in front of pituitary. No peristalsis seen.

F. Two points stimulated in the same brain. In nine cats of the series, two points were stimulated in the same brain, making possible the comparison of thresholds for inhibition for different regions with the disturbing factor of individual variability eliminated. For example, in cat 10, stimulation of the basis pedunculi resulted in inhibition when the coil was at 10 cm. Stimulation of another point in the internal capsule dorsal to the lateral geniculate body elicited no change in peristalsis even when the secondary coil was at 0. In cat 15, two points in the preoptic area had thresholds of 10 and 9 cm. In cat 14, the electrode in the internal capsule and the one in the infundibular stalk had about the same threshold. In cat 9, inhibition was obtained on hypothalamic stimulation at coil 17, while coil 10 was the threshold for a point more dorsally placed. The results indicate that the hypothalamus has the lowest threshold and that the coil distance required for inhibition is similar for internal capsule, preoptic area and infundibulum.

The most convincing of this group of experiments were the three in which two points were stimulated along a single electrode, one in the hypothalamus and one in the thalamus (fig. 5). The location of the points and the thresholds were:

Cat 21—(*a*) in nucleus hypothalamicus ventromedialis. Inhibition at coil 14. (*b*) on surface of anterodorsal nucleus of thalamus. Inhibition at coil 11½.

Cat 26—(*a*) in lateral hypothalamic area at level of mammillary body. Inhibition at coil 14. (*b*) in nucleus lateralis pars posterior of thalamus, 2½ mm. from midline. Inhibition at coil 9 (fig. 5).

Cat 27—(*a*) in field H_1 of Forel. Inhibition at coil 15. (*b*) about 1 mm. dorsal to surface of thalamus, in region of pulvinar. Inhibition at coil 9.

The vigorous responses to stimulation with the hypothalamic electrodes have been adequately described. Stimulation of the thalamus, using coil distances of 15 to 12 cm. caused no change in peristalsis and only occasional slight dilatation of the pupils. Stronger stimulation of the thalamus that resulted in inhibition of the gut also produced marked dilatation of the pupils and rapid, very shallow respiration, followed after 20 to 30 seconds by a very violent clonic convulsion that lasted for a minute or two and was accompanied by profuse salivation. The fit proceeded whether the stimulation was continued or not. If the current was strong enough, the stimulus could be stopped after 10 seconds and the convulsions would follow. This mechanism was easily fatigued and would only repeat at reduced coil distances. As a rule, the gastro-intestinal inhibition began with the onset of the fit and not when stimulation was started. It lasted during the convulsions and also for a minute or two afterward, a rather brief after-inhibition following such a violent reaction. In cat 21, inhibition occurred without a fit.

Convulsions have also been observed in eight cats during very strong stimulation of the hypothalamus (secondary coil set at 4 cm.). These convulsions differed from those elicited from the thalamus in being much less clonic in character, in coming on at once at the beginning of stimulation and in stopping immediately on removal of the stimulus. It should be emphasized, however, that nothing resembling a convulsion ever occurred

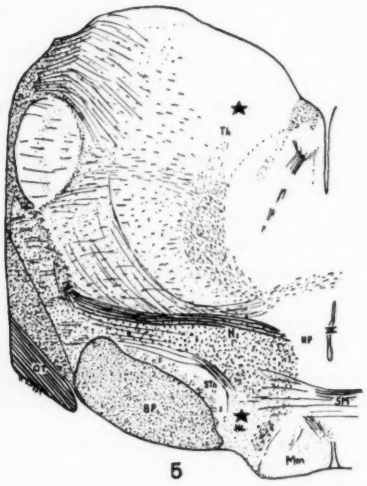


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Fig. 5. Transverse section through the diencephalon at the level of the mammillary bodies. Stimulation of two points, one in the lateral hypothalamic area and one in the thalamus.

List of abbreviations: *AC*, anterior commissure; *AP*, anterior pituitary; *BP*, basis pedunculi; *CC*, corpus callosum; *CN*, caudate nucleus; *dHa*, dorsal hypothalamic area; *E*, entopeduncular nucleus; *F*, fornix; *FP*, nucleus filiformis principalis; *GlP*, globus pallidus; *HL*, lateral hypothalamic area; *HP*, nucleus hypothalamicus posterior; *Hvl*, nucleus hypothalamicus ventrolateralis; *Hvm*, nucleus hypothalamicus ventromedialis; H_1, H_1 field of Forel; *I*, infundibular stalk; *IC*, internal capsule; *LV*, lateral ventricle; *MFB*, medial forebrain bundle; *Mm*, medial mammillary nucleus; *MT*, mammillo-thalamic tract; NH_1, nucleus of H_1 field of Forel; *OC*, optic chiasma; *OLS*, olfacto-septal fibers; *OT*, optic tract; *Pd*, nucleus hypothalamicus periventricularis dorsalis; *Pu*, putamen; *Pv*, nucleus hypothalamicus periventricularis ventralis; *S*, septum pellucidum; *SM*, supramammillary commissure; *STh*, subthalamic nucleus of Luys; *Th*, thalamus; *III*, third ventricle.

with the strengths of current used in eliciting from the hypothalamus an inhibition of the gastro-intestinal musculature and the other sympathetic and somatic responses described in this paper.

A number of observations have accumulated on the effects of pain on gastro-intestinal motility. Pinching of the paw caused struggling, crying, rapid respiration, dilatation of the pupils and sometimes erection of hair

It had no effect on peristalsis in two out of three normal animals and in nine out of eleven operated animals studied.

Discussion. In recent years, the brain has been stimulated in unanesthetized animals by Pachon and Delmas-Marsalet (7), Hess (8) and Mussen (9).

The use of the x-ray after a barium meal to study gastro-intestinal motility is the most satisfactory, since no operative procedure on the abdomen is required and changes in tone and motility can be clearly seen.

Our results indicate that there is a center in the hypothalamus which inhibits gastro-intestinal motility. Just how much of the hypothalamus is concerned in this inhibitory mechanism we cannot say for certain. The lateral hypothalamic area is surely involved, since stimulation of this region always results in gastro-intestinal inhibition. Furthermore, it is known that the responses of the pupils, the bladder, blood pressure and respiration are most marked on stimulation of this region (1, 2, 3) and since no inhibition occurs unaccompanied by a marked sympathetic discharge, it is safe to assume that the single center is concerned in all of these responses. The infundibulum may be eliminated from consideration on the basis of the negative results of the two experiments described above. The possibility, however, that the medial hypothalamus is part of the inhibitory center cannot be excluded, since the only point stimulated in this region (the ventromedial hypothalamic nucleus in cat 21) did produce abolition of digestive motility. Stimulation of the supramammillary commissure in one experiment caused inhibition, suggesting that these fibers are part of the pathway from the hypothalamus to the gut.

All components of the response to stimulation of the hypothalamus do not have the same threshold and the gastro-intestinal inhibition seems to have the highest of any. Dilatation of the pupils, erection of hair, increases in rate and depth of respiration, urination, and struggling have resulted from stimulation at coil distances too great to yield cessation of peristalsis.

Observations on the effects of subcortical stimulation on the motility of the alimentary tract have been made by some other investigators (10, 11, 12, 13, 14, 15) with results differing considerably from our own.

One by one, the changes that Cannon (16) has observed in his emotionally excited cats are being found to follow electrical stimulation of the hypothalamus. Karplus and Kreidl, beginning in 1909, investigated these responses. In a summary of their work, Karplus (17) lists pupillary dilatation, rise in blood pressure, contraction of the bladder, secretion of sweat, tears and saliva, and occasional crying as responses to hypothalamic stimulation. Recently their findings have been confirmed and more precise information on localization obtained (1, 2, 3). In the present paper, with waking animals, we have added erection of hair, the intense expression and violent movements typical of strong emotional reactions,

even including snarling and baring of the claws as well as inhibition of gastro-intestinal motility to the growing list. The similarity between the response of a cat to a barking dog and its response to faradic stimulation of the lateral hypothalamic area is now perfectly clear. This is in full agreement with the conclusions reached by Bard (18) and points to the hypothalamus as the center responsible for the integration of the reaction pattern characteristic of intense emotional excitement (19).

CONCLUSIONS

1. Electrical stimulation of the lateral hypothalamic area in the unanesthetized cat causes cessation of peristalsis and loss of tone of the stomach and small intestine.

2. Stimulation of the thalamus, internal capsule, anterior commissure, septum pellucidum and infundibular stalk with the same strength of current produces no change in gastro-intestinal motility.

3. No increases in tone or motility were observed at any time from any of the various points stimulated in the brain.

4. These results are discussed in relation to the hypothesis that the hypothalamus is the integrating center for emotional reactions.

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